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14. ABSTRACT This was a two-year randomized trial of the effects of oral contraceptives on bone mass and stress fracture incidence among 150 female competitive distance runners of ages 18-26 years. The Coordinating Center was at Stanford University and bone mass was measured at five sites: Massachusetts General Hospital, University of California Los Angeles, University of Michigan, Stanford University/Palo Alto VA Medical Center, and Helen Hayes Hospital in West Haverstraw NY. In addition to a publication from baseline data, two manuscripts have been accepted for publication. One manuscript, "The effect of oral contraceptives on bone mass and stress fractures in female runners," concludes that oral contraceptives may reduce the risk for stress fracture, but our data are inconclusive. The second manuscript, "Risk factors for stress fracture among young female cross-country runners," found that a history of stress fractures, lower bone mass, lower dietary calcium intake, younger chronological age, younger age at menarche, and possibly a history of irregular menstrual periods were associated with an increased risk. Another manuscript, "The effect of oral contraceptives on body weight and body composition in young female runners" will be submitted for publication shortly. Two other manuscripts are in preparation.						
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(5) INTRODUCTION

Highly trained female athletes may experience loss of menses because of their participation in intense physical activity. Previous cross-sectional research has shown that women with exercise-induced menstrual irregularities have a significantly higher frequency of stress fractures and low bone mass than normally menstruating controls. Longitudinal studies suggest that these women are losing bone mass over time. Low serum estrogen levels are believed to be a principal cause of the bone loss. If so, re-establishing normal estrogen levels in these women should prevent or retard bone loss and decrease the incidence of stress fracture. This study was a two-year randomized trial of the effect of oral contraceptives on bone mass and stress fracture incidence among 150 female cross country runners in the age range 18-26 years. The Coordinating Center is at Stanford University and bone mass was measured at five sites: the Massachusetts General Hospital, the University of California Los Angeles, the University of Michigan, Stanford University/Palo Alto VA Medical Center, and the Helen Hayes Hospital in West Haverstraw, NY. Athletes were recruited mostly from the areas around these five clinical sites.

(6) BODY

Work Previously Reported

One hundred fifty eligible female runners were randomized, of whom 124 (83%) attended at least one follow-up appointment and 96 (64%) attended both, and at average of 14.4 and 26.6 months, respectively. Three additional women provided information on stress fracture occurrence for an average of 7.9 months after baseline. Data collection has

been completed, the data have been cleaned and prepared for statistical analysis, statistical analyses have been undertaken, and manuscripts have been written.

One manuscript from baseline data, “Disordered eating, menstrual irregularity, and bone mineral density in female runners,” was published in *Medicine & Science in Sports & Exercise* in 2003. Two other papers are scheduled for publication in the same journal in September 2007: “The effect of oral contraceptives on bone mass and stress fractures in female runners” and “Risk factors for stress fracture among young female cross-country runners.” Copies of these articles are appended.

Work Accomplished During Period of Final No-cost Extension (November 2006 – July 2007).

As part of a final no-cost extension granted in late 2006, four secondary analyses of the data already collected were proposed. Each of the four analyses will be discussed in turn.

No-Cost Extension Objective (1): Complete the analysis and prepare a manuscript on the question of whether use of oral contraceptives is associated with changes in weight and/or body composition.

A manuscript, “The effect of oral contraceptives on body weight and body composition in young female runners,” has undergone review by all authors, will undergo slight revision as a result of their suggestions, and will be submitted to *Exercise & Science in Sports & Medicine* shortly. A copy of the penultimate draft of this manuscript is appended.

No-Cost Extension Objective (2): Analyze the data to identify what other factors (e.g., training regimen, diet) are related to changes in weight and/or body composition.

We summarize below the results of our analyses. We plan to prepare a manuscript shortly.

Factors Associated with Changes in Body Composition and Weight

Background

Weight, lean mass, and fat mass are of concern to runners because of their potential effects on health and running performance. It is well known that in both young women and men, physical activity, including running, decreases body mass index as well as more direct measures of fat mass. At least one study in runners has documented that low fat mass is associated with faster running speed, and several studies have reported that the more miles run per week, the lower the fat mass. In healthy, active young women, weight training and physical activity in general have been reported to increase lean mass, but little is known of factors than influence lean body mass specifically in runners, who are already very physically active.

In young people in the general population, fat mass and especially lean mass are independently associated with measures of bone mass, but these associations have not been well studied in runners. The effects of changes in weight and body composition on the propensity of runners to develop eating disorders are also not known.

In an analysis described under no-cost extension objective (1), we reported that use of the oral contraceptive Lovral was associated with gain in lean mass but not fat mass among eumenorrheic but not among oligomenorheic/amenorrheic runners. This report considers several other aspects of changes in weight and body composition in runners. First we examine the effects of training-related activities and dietary constituents on changes in lean mass, fat mass, percent body fat, and weight. Then we examine the

associations between changes in weight and body composition on daily calories consumed, score on an eating disorder inventory, and bone mass.

Results

Descriptive statistics

In this project 124 women seen at baseline and at least one follow-up visit are included in the analyses concerned with weight, and 123 women are included for analyses concerned with lean mass, fat mass, and percent body fat. In some analyses numbers are lower because of missing data. Table 1 (pages 14-15) indicates that the mean age at baseline of the women was 22.0 years, and the mean body mass index was 21.2 kg/m^2 . Mean caloric intake was 2318 kcal/day. Participants ran for an average of 35 miles per week and lifted weights for an average of 64 minutes per week in the past year

Training-related variables: Table 2 (page 16) shows that runners who ran more miles per week and those who increased their number of miles run per week during the period of the study tended to lose weight, fat mass, and percent fat mass. Miles run per week had little effect on lean mass. In contrast, greater time spent lifting weights and an increase in the amount of time spent lifting weights were associated with an increase in lean mass, but had little effect on weight, fat mass, or percent body fat. These trends held regardless of menstrual status (oligo/amenorrheic [fewer than 10 menstrual periods in past year] versus eumenorrheic [10 or more menstrual periods in past year]). These trends were also examined according to treatment assignment in the randomized trial, and significant ($p = 0.006$) effect modification was found in one instance: the annual rate of change in kilograms of lean mass per minute weekly weight lifting at baseline was

greater in those randomly assigned to the oral contraceptive group ($b = 0.0135 \pm 0.0031$) than among those assigned to the control group ($b = 0.0034 \pm 0.0028$).

Dietary constituents: Little change occurred over the period of the study in the amount of specific dietary constituents consumed by most of the runners, so we were unable to examine the effect of changes in diet (other than total daily calories consumed, as discussed below). Instead, we examined changes in weight and body composition in relation to baseline dietary characteristics. We ran regressions of changes in weight and body composition on baseline values of several key dietary components, including daily intake of fiber, vegetable protein, animal protein, fat, calcium dairy products, iron, sodium, vitamin C, total soda, diet soda, regular soda, wine, beer, liquor, and number of “standard” alcoholic drinks, controlling for clinical site, randomization assignment, and total average calories consumed per day at baseline. No statistically significant associations with weight or body composition were found at $p < 0.05$, and only two were found at $p < 0.10$: vegetable protein with fat mass, and vegetable protein with percent body fat (data not shown). These two associations are easily attributable to chance, given the large number of associations examined. It may be seen in Table 1 that the runners had low consumption of certain dietary constituents of interest, such as soft drinks and alcohol beverages, so we had little statistical power to examine their effects.

Sufficient variation over time occurred in total calories consumed per day that we were able to examine associations of change in average daily calories consumed and changes in weight and body composition. Of some interest was an initially counter-intuitive, although not statistically significant, association between increases in weight, fat mass, and percent body fat and a decrease in total daily calories consumed over the

period of the study. For instance, the regression of change in percent body fat during the study period on change in kilocalories consumed per day during the study period was - 0.0010 per year ($p = 0.07$). Upon detailed examination, we found that an increase in percent fat during the *first* year was strongly associated with a reduction in daily kilocalories consumed during the *second* year of follow-up (decrease in second year of kilocalories per percent increase in body fat during first year = -105.6, $p = 0.008$). In other words, those who gained body fat during the first year subsequently reduced the amount of calories consumed during the second year. In contrast, little association was seen between percent body fat gained during the *second* year and daily kilocalories reported at the end of the *first* year (gain in kilocalories in first year per percent increase in body fat during second year = 23.3, $p = 0.54$)

Eating disorder score: We found positive associations between changes in weight, fat mass, and percent fat and rise in the total eating disorder score, mainly in oligo/amenorrheic runners. This association was a result of both an increase in the eating disorder score among those who gained weight and a decrease in the eating disorder score among those who lost weight. Detailed examination again indicated that the change in eating disorder score followed a first year change in weight or fat, and that weight or fat change during the second year was not at all associated with the eating disorder score at the end of the first year. Thus, weight and fat gain tend to result in caloric restriction in both eumenorrheic and oligo/amenorrheic runners, but appear to create anxiety, as indicated by a rise in eating disorder score, mainly among oligo/amenorrheic runners. Of the three subscales of the eating disorder inventory that were included in this study

(drive-for-thinness, bulimic tendency, and body dissatisfaction), the above results were mainly driven by the body dissatisfaction subscale

Bone mineral density (BMD): It is difficult to state with certainty the direction of associations between changes in weight and body composition and changes in BMD, but it seems most likely that changes in weight and/or body composition lead to changes in BMD or that some third factor affects weight and/or body composition and BMD. Therefore, we considered changes in BMD as the dependent variable and changes in weight and body composition as the independent variables.

Baseline weight and body composition were not related to changes in spine BMD (data not shown), but *changes* in weight and all three body composition measures were associated with changes in spine BMD (Table 3, pages 17-18). In other words, as weight, fat mass, percent body fat, and lean mass increase, so does spine BMD. When we divided the runners into those who were eumenorrheic (10+ menstrual cycles in the past year) and those who were amenorrheic or oligomenorrheic (fewer than 10 menstrual cycles in the past year), it may be seen that for weight, fat mass, and percent body fat, the positive associations occur in oligo/amenorrheic women, but not in eumenorrheic women, who had substantially higher BMD at baseline anyway. The association for lean mass is stronger for eumenorrheic women than oligo/amenorrheic women. Among the eumenorrheic women, the annual increase in BMD (g/cm^2) per average annual kilogram change in lean mass was stronger for those randomly assigned to the oral contraceptive group ($b = 0.0093 \pm 0.0031$) than those assigned to the control group ($b = 0.0015 \pm 0.0021$) ($p = 0.04$ for effect modification.)

Table 3 also shows that, again, associations between increases in weight and body composition measures and changes in hip BMD are apparent for oligo/amenorrheic women, but not for eumenorrheic women. Only for change in lean mass is there a suggestion of a slight positive association with change in BMD for eumenorrheic women. Weak negative associations were seen between lower baseline weight and change in hip BMD in all women ($p = 0.10$) and in oligomenorrheic women ($p = 0.04$) and between lower baseline fat mass and hip BMD in all women ($p = 0.06$), but otherwise no notable associations between baseline measures and changes in BMD were seen (data not shown).

Conclusions

These data suggest that among young female long-distance runners in the age range 18-26 years:

- (1) Those who want to reduce weight and fat can do so by running more miles per week. Lifting weights does not appear to lead to a reduction in weight and fat mass.
- (2) Those who want to increase lean mass can do so by increasing their weekly duration of weight lifting. Increasing number of miles run per week does not appear to increase lean mass.
- (3) Specific dietary constituents, as measured in this study and consumed by these runners, do not appear to affect changes in weight or body composition.
- (4) Those who gain weight and fat subsequently tend to restrict their total caloric intake.
- (5) Oligo/amenorrheic runners who gain weight and fat subsequently tend to have higher eating disorder inventory scores.

(6) Gains in weight, fat mass, percent body fat, and lean mass are associated with increases in bone mineral density in the spine and hip, but only among those who are initially oligo/amenorrheic. Thus, for the sake of bone health, it is desirable for oligo/amenorrheic runners to gain weight, fat, and lean mass.

Limitations

In addition to limitations in the study as a whole that are discussed elsewhere, these analyses were somewhat hindered by having only 1-2 follow-up visits about a year apart, making it difficult to sort out time sequences for some variables, such as changes in body composition and changes in bone mineral density; the relatively short, two-year duration of the study; the lack of change in diet over the two-year period; and the low consumption of certain beverages such as soda and alcohol.

Table 1. Characteristics of study population at baseline.

Characteristic	Mean	Standard Deviation
Age (years)	22.0	2.6
Weight (pounds)	128.9	14.8
Height (inches)	65.3	2.5
Body mass index (kg/m^2)	21.2	1.9
Lean mass (kg)	41.9	4.5
Body fat (kg)	13.4	3.9
Body fat percentage	23.1	5.3
Hip bone mineral density (g/cm^2)	0.889	0.119
Spine bone mineral density (g/cm^2)	0.988	0.110
Whole-body bone mineral content (g)	2174	293
Dietary intake		
Kilocalories/day	2318	950
Fiber (g/day)	30.3	18.3
Fat (g/day)	48.7	29.2
Protein (g/day)	92.6	39.8
Regular soda (ounces/day)	1.4	3.5
Diet soda (ounces/day)	4.3	10.4
Wine (ounces/day)	0.4	0.8

Beer (ounces/day)	1.7	3.5
Liquor (ounces/day)	0.08	0.17
Number of standard drinks/day	0.28	0.42
Vegetable protein (g/day)	42.9	25.2
Animal protein (g/day)	50.0	27.2
Dietary calcium (mg/day)	1364	683
Iron (mg/day)	23.0	15.8
Vitamin C (mg/day)	274	183
Total eating disorder score*	12.0	12.5
Average distance run in past year (miles/week)	34.7	11.2
Average time lifting weights in past year (min/wk)	63.9	51.1
Amenorrheic at baseline	8.1%	
Oligomenorrheic at baseline	25.0%	
Eumenorrheic at baseline	66.9%	

*Eating Disorder Inventory 0-69; 0=least disordered, 69=most disordered, Garner and Olmstead (1984)

Table 2. Adjusted^a annual rates of change in weight, fat mass, percent fat mass, and lean mass by baseline training characteristics and annual change in training characteristics

Training characteristic	Weight (pounds ± SE)	Fat mass (kg ± SE)	Body fat (% ± SE)	Lean mass (kg ± SE)
Miles run per week (N = 114/113) ^b				
At baseline	-.0700±.0318*	-.0293±.0137*	-.0377±.0189*	-.0029±.0087
Annual increase	-.2072±.0633**	-.0675±.0281*	-.0987±.0389*	.0023±.0179
Minutes lifted weights per week (N = 102)				
At baseline	.0075±.0090	-.0020±.0038	-.0046±.0052	.0068±.0021**
Annual increase	.0152±.0180	-.0001±.0076	-.0017±.0105	.0099±.0042*

^aAnnual rates of change are adjusted by multiple linear regression for clinical site and treatment assignment in the randomized trial. In addition, annual increases are adjusted for baseline measures, and baseline measures are adjusted for annual increases.

^bFirst number refers to those included in the analyses concerned with weight, second number to those included in the analyses concerned with body composition.

*p<0.05, rate of change differs from 0.

** p<0.005, rate of change differs from 0.

Table 3. Adjusted^a annual rates of change in spine and hip bone mineral density (BMD) by annual rate of change in weight, fat mass, percent body fat, and lean mass, by menstrual status

Annual change in weight/composition measure	Annual change in spine BMD (g/cm ²)	Annual change in hip BMD (g/cm ²)
All women (N = 124/123)^b		
Weight (pounds)	.0011±.0003**	.0007±.0003*
Fat mass (kg)	.0023±.0008**	.0017±.0007*
Body fat (%)	.0015±.0006*	.0010±.0006
Lean mass (kg)	.0029±.0011*	.0035±.0010**
Eumenorrheic women^b (N = 83)		
Weight (pounds)	.0005±.0006	.0001±.0006
Fat mass (kg)	-.0007±.0013	-.0003±.0013
Body fat (%)	-.0012±.0009	-.0004±.0009
Lean mass (kg)	.0042±.0017*	.0025±.0018

Table 3 (continued)

Annual change in weight/composition measure	Annual change in spine BMD (g/cm²)	Annual change in hip BMD (g/cm²)
Oligo/amenorrheic women^d (N = 41/40)^b		
Weight (pounds)	.0010±.0003**	.0008±.0003*
Fat mass (kg)	.0035±.0009**	.0032±.0010**
Body fat (%)	.0033±.0008**	.0024±.0009*
Lean mass (kg)	.0016±.0015	.0038±.0015*

^aAnnual rates of change are adjusted by multiple linear regression for clinical site, treatment assignment in the randomized trial, and baseline weight or body composition measure.

^bFirst number refers to those included in the analyses concerned with weight, second number to those included in the analyses concerned with body composition.

^cEumenorrheic women reported having had 10 or more menstrual cycles during the year before baseline.

^dOligomenorrheic and amenorrheic women reported having had fewer than 10 menstrual cycles during the year before baseline.

*p<0.05, rate of change differs from 0.

**p<0.005, rate of change differs from 0.

No-Cost Extension Objective (3): Analyze the data to see whether beverage consumption affects bone mass and risk of stress fracture.

We present the results of these analyses below. We are preparing a manuscript that combines both the results below on beverages and the results on solid and semi-solid foods (not an official part of the no-cost-extension). In the analysis of solid and semi-solid foods, there is a suggestion of a beneficial effect against stress fracture from calcium, Vitamin D, protein, and dairy products, although none of these associations was statistically significant in Cox regression models. Below is a summary of our results on beverages:

Beverage Consumption and Changes in Bone Mass and Stress Fracture Occurrence

Background

Certain beverages are believed to affect bone growth, bone loss, and fracture risk. Milk has been linked to increased bone mass and density and lower fracture risk in some but not all studies. Coffee, caffeine, soda (particularly cola-type sodas), and alcohol have been linked to bone loss and increased fracture in some but not all studies. Most studies of beverage intake and bone in women have focused on adolescent or post-menopausal populations. We analyzed data on the effects of soda, caffeinated coffee, caffeine, soy milk, dairy milk, and alcohol on fracture risk and changes in bone mass and density in young adult women runners.

Results

Of the 150 women randomized in the study, 125 women provided data on beverage consumption and also provided follow-up data, and only these women were included in

these analyses. Table 1 (page 24) displays descriptive statistics on the consumption of soda, caffeinated coffee, caffeine (from beverages), soy milk, dairy milk, and alcohol at baseline. Most of the distributions were right skewed, with low to moderate average consumption and few heavy consumers. The average milk intake (dairy milk consumed as a beverage) was 8 ounces per day; the majority of dairy milk consumed was skim milk. The average caffeinated coffee intake was less than 8 ounces per day, and average caffeine was 150 mg per day. The women drank less than 8 ounces of soda per day, mostly from diet soda rather than sweetened soda. The average alcohol intake was low at less than 3 standard drinks per week.

Beverages and stress fracture

Eighteen runners had at least one stress fracture during the study in the tibia, foot, femur, or pelvis. We explored the relationship between baseline beverage consumption and stress fractures using two approaches: Table 2a (page 25) shows hazard ratios for beverages treated as continuous variables; Table 2b (page 26) shows hazard ratios for the top quartiles of beverage consumption (compared with the lower three quartiles). Results were similar with both analyses. After adjusting for clinical site, treatment group assignment, menstrual status at baseline, spine bone density, age, and stress fracture history in Cox proportional hazards models, we found that only intakes of skim milk and total dairy milk were significantly related to fracture. Every additional cup of skim milk consumed per day was associated with a 62% reduced fracture risk; every additional cup of any dairy milk consumed per day was correlated with a 57% reduction in risk. Levels

of low-fat and whole milk consumption were low, which may explain the failure to find effects with these specific beverages.

There were insufficient numbers at truly “high” levels of consumption to evaluate the effects of high levels of caffeine, coffee, soda, and alcohol (which have previously been associated with increased fracture risk). Women in the top quartile of consumption of these beverages were generally consuming what would be considered light to moderate amounts. At these levels, we found no evidence of harmful effects for these beverages. However, we cannot rule out harmful effects at higher levels of consumption.

Results did not vary by menstrual group (amenorrheic, oligomenorrheic, and eumenorrheic) or by disordered eating status (yes/no). We found no evidence of confounding by weight, caloric intake, or training, so these variables were not included in the final models.

We found similar results when we repeated all analyses using average beverage consumption over the entire study period rather than baseline beverage consumption and when we modeled consumption as a time-dependent (time-changing) variable. In these analyses, hazard ratio estimates were similar but confidence intervals were slightly wider reflecting variability over the study period.

Beverages and changes in bone mineral content and density

Table 3 (pages 27-28) shows the relationship between beverage consumption and changes in bone mineral content (BMC) and bone mineral density (BMD). Annual rates of change were obtained from linear mixed models, adjusted for clinical site, age, annual menses, and treatment assignment in the randomized trial. Skim milk and total milk

predicted changes in hip BMD and whole body BMC. Every additional cup of skim milk consumed per day increased the rate of change in hip BMD by $.00263 \pm .00089$ g/cm² and increased the rate of change in whole body BMC by 5.2 ± 2.2 grams per year. Estimates were similar for total dairy milk.

Caffeine and caffeinated coffee predicted positive changes in spine and hip BMD.. It is possible that women are consuming a significant amount of dairy milk in their coffee, which could explain this effect. However, caffeine and caffeinated coffee were not associated with changes in whole body BMC (as seen with milk).

None of the other beverages were significantly associated with bone changes. For sweetened soda and alcohol, the rates of change tended to be negative, but this was not consistent at all bone sites.

Trends were very similar among the different menstrual groups, so we did not stratify on menstrual group for the final models. There was no evidence of confounding by weight, caloric intake, or training, so these variables were not included in the final models.

Conclusions

Skim milk and total dairy milk are protective against fracture and are associated with longitudinal increases in hip BMD and whole body BMC. No other beverages appear associated with fracture (either harmful or protective), at least at the levels being consumed by these women. Surprisingly, caffeine and coffee are associated with positive changes in spine and hip BMD. This finding could be due to chance, unmeasured confounding, or could reflect the addition of dairy products to coffee. No other beverages

were associated with changes in bone density or mass, but the levels of consumption may have been too low to detect effects.

Limitations

Our questionnaire did not distinguish between cola types of soda and other types. This may have obscured our ability to see the effects of cola-type sodas, which may be more harmful for bone. The women in our study generally reported only light to moderate consumption of soda, coffee, and alcohol, so we cannot rule out harmful effects at higher levels of consumption.

Table 1. Descriptive statistics for selected beverages, baseline consumption (n=125).

Beverage	Mean	SD	Median	Min.	Max.
Sweetened soda, ounces/day	1.4	3.5	0	0	30.0
Diet soda, ounces/day	4.2	10.3	0	0	54.0
Total soda, ounces/day	5.6	10.6	1.4	0	54.0
Caffeinated coffee, ounces/day	6.3	12.1	0.6	0	72.0
Caffeine, mg/day	140	233	33	0	1350
Soy milk, ounces/day	1.8	4.6	0	0	36.0
Skim milk, ounces/day	5.7	8.5	2.6	0	56.0
Low-fat milk, ounces/day	1.7	4.5	0	0	20.0
Whole milk, ounces/day	0.1	0.3	0	0	2.6
Total dairy milk, ounces/day	9.3	9.1	6.9	0	56.0
Wine, ounces/day	0.4	0.8	0	0	3.9
Beer, ounces/day	1.7	3.5	0.5	0	30.0
Liquor, ounces/day	0.1	0.2	0	0	0.9
Total alcohol, standard drinks/day	0.3	0.4	0.1	0	2.6

Table 2a. Adjusted* hazard ratios (and 95% confidence interval) for associations between beverage consumption and stress fractures.

Beverage	Hazard Ratio (95% CI)
Sweetened soda, cups/day	0.43 (0.02, 11.15)
Diet soda, cups/day	0.93 (0.53, 1.62)
Total soda, cups/day	0.91 (0.52, 1.57)
Coffee, cups/day	0.94 (0.63, 1.40)
Caffeine, 100 mg/day	0.95 (0.73, 1.25)
Soy milk, cups/day	1.27 (0.52, 3.12)
Skim milk, cups/day	0.38 (0.16, 0.89)**
Low-fat milk, cups/day	0.86 (0.34, 2.17)
Whole milk, cups/day	Insufficient data to estimate
Total dairy milk, cups/day	0.43 (0.20, 0.89)**
Wine, ounces/day	0.09 (0.01, 1.40)
Beer, ounces/day	0.83 (0.52, 1.34)
Liquor, ounces/day	2.32 (0.05, 103.05)
Total alcohol, standard drinks/day	0.21 (0.01, 6.38)

*Adjusted for clinical site, treatment group assignment, menstrual status at baseline, spine bone density, age, and stress fracture history.

**p<.05

Table 2b. Adjusted* hazard ratios (and 95% confidence interval) for associations between high consumption of beverages (top quartile) and stress fractures.

Beverage	Hazard Ratio (95% CI)
Sweetened soda, ≥ 1 oz/day	0.75 (0.18, 3.07)
Diet soda, ≥ 2 oz/day	0.45 (0.07, 4.43)
Total soda, ≥ 5 oz./day	0.69 (0.12, 4.10)
Coffee, ≥ 8 ounces/day	0.69 (0.12, 4.10)
Caffeine, ≥ 181 mg/day	0.69 (0.12, 4.10)
Soy milk, ≥ 1 oz/day	0.99 (0.23, 4.22)
Skim milk, ≥ 8 ounces/day	0.08 (0.01, 0.79)**
Low-fat milk, > 0.05 ounces/day	0.69 (0.17, 2.78)
Whole milk, > 0 ounces/day	0.84 (0.19, 3.67)
Total dairy milk, ≥ 8.5 ounces/day	0.14 (0.02, 0.79)**
Wine, ≥ 0.4 ounces/day	0.09 (0.01, 1.04)
Beer, ounces/day, ≥ 1.7 ounces/day	0.47 (0.09, 2.32)
Liquor, ≥ 0.1 ounces/day	0.89 (0.20, 3.93)
Total alcohol, ≥ 0.4 standard drinks/day	0.26 (0.03, 2.53)

*Adjusted for clinical site, treatment group assignment, menstrual status at baseline, spine bone density, age, and stress fracture history.

**p<.05

Table 3. Adjusted* annual rates of change in spine, hip, and whole body mineral density (BMD) and whole body bone mineral content (BMC) and skeletal area by beverage consumption.

Beverage	Spine BMD (g/cm ² /year ± SE)	Total hip BMD (g/cm ² /year ± SE)	Whole body BMC (g /year ± SE)
Sweetened soda, rate of change per additional cup/day	-.00032 ± .00195	.00140 ± .00184	-3.1 ± 4.4
Diet soda, per additional cup/day	.00067 ± .00086	.00032 ± .00081	1.3 ± 1.9
Total soda, per additional cup/day	.00053 ± .00081	.00052 ± .00076	0.5 ± 1.8
Coffee, per additional cup/day	.00146 ± .00066	.00167 ± .00063 ^{**}	-0.4 ± 1.5
Caffeine, per additional 100 mg/day	.00098 ± .00043	.00111 ± .00041 ^{**}	-0.2 ± 1.0
Soy milk, per additional cup/day	-.00099 ± .00202	.00081 ± .00191	1.1 ± 4.5

Skim milk, per additional cup/day	.00096 ± .00096	.00263 ± .00089 [§]	5.2 ± 2.2
Low-fat milk, per additional cup/day	-.00149 ± .00200	.00053 ± .00019	1.2 ± 4.5
Total dairy milk, per additional cup/day	.00064 ± .00093	.00268 ± .00086 [§]	5.1 ± 2.1
Wine, per additional ounce/day	-.00042 ± .00093	.00205 ± .00146	-2.0 ± 3.5
Beer, per additional ounce/day	-.00025 ± .00031	.00006 ± .00030	0.2 ± 0.7
Liquor, per additional ounce/day	-.01220 ± .00812	.00359 ± .00771	-0.7 ± 18.5
Total alcohol, per additional standard drink/day	-.00299 ± .00284	.00030 ± .00269	-0.1 ± 6.5

*Annual rates of change are estimated from linear mixed models, adjusted for clinical site, age, annual menses, and treatment assignment in the randomized trial.

| p<.05, rate of change differs from 0.

** p<.01, rate of change differs from 0.

‡ p<.10, rate of change differs from 0.

§ p<.005, rate of change differs from 0.

No-Cost Extension Objective (4) analyze the data to try to see what factors are associated with spontaneous return of regular menses among women with irregular periods.

As expected, numbers were too small for definitive results, but we summarize our tentative results below. We have not yet decided whether these results merit publication.

Factors Associated with Return to Menses

Background

The female athlete triad, a syndrome consisting of disordered eating, menstrual irregularity, and osteopenia/osteoporosis, is of concern among female athletes. Because of the interrelationships among dietary, exercise, and behavioral characteristics, it is difficult to determine what factors are responsible for cessation of menses. It is also unclear what factors contribute to resumption of normal menstrual function in amenorrheic and oligomenorrheic athletes. This analysis sought to identify factors associated with the resumption of menses in those oligo/amenorrheic at baseline.

The study population for these analyses consisted of those who were amenorrheic or oligomenorrheic at baseline, had taken oral contraceptive pills for less than 6 months during the study, and had at least one follow-up visit. We approached the analysis as a case-control study, in which we divided this group into two subgroups based on the menstrual pattern at the final follow-up visit. If the participant had resumed a normal menstrual pattern, she was considered to have “spontaneously resumed” her periods. If she remained with irregular

menstrual patterns at study visit 3, she was considered to have “remained irregular” Factors of interest were analyzed at baseline, the first follow-up visit, and the second follow-up visit. In addition to the three points, variables were also analyzed based on the changes between follow-up visits. To identify factors associated with resumption of menses, means of the variables of interest were compared using ANOVA tests. A level of $p<0.10$ was considered statistically significant.

Results

Of the 25 runners included in the analysis, 14 spontaneously regained (SR) normal menstrual function by their final follow-up visit and 11 remained irregular (RI). Table 1 (page 33) shows characteristics of women in the two groups.

Table 2 (pages 34-36) shows that the SR group was more likely to have had a decrease in eating disorder inventory- anorexia nervosa subscale score between baseline and the first follow-up visit than the RI group. For the total eating disorder inventory scale, the SR group had a trend towards decreasing scores at each follow-up visit, while the RI group did not. The SR group consumed more total daily calories than the RI group at baseline. The SR group continued to consume more calories per day at each follow-up compared to the RI group, although these differences were not statistically significant. The SR group also had a greater increase in percentage of calories from fat than the RI group between follow-up visits 1 and 2; this trend, although not statistically significant, appeared between baseline and follow-up visit 2 as well. The SR group ran more miles per week than the RI group at baseline and first follow-up, but the SR group

showed a trend towards greater decrease in mileage over the study period than the RI group. Finally, there was a non-significant trend towards an increase in weight over time in the SR group, as compared to a slight decrease in weight for the RI group.

Menstrual resumption was also analyzed as a function of energy balance. A calculation for energy balance was created as the kilocalories consumed minus the kilocalories expended in running. While the SR group appeared to have a larger energy “reservoir” at each time point, the findings were not statistically significant.

Several factors not included in the table were examined but did not yield any notable findings, including lean mass, fat mass, and the eating disorder inventory- bulimia nervosa subscale.

Conclusions

Despite the small numbers of participants, increased intake of calories and dietary fat was associated with the resumption of normal menses. Additionally, a decrease in eating disorder behaviors, specifically anorexic behaviors, was linked to the resumption of normal menses.

Surprisingly, the athletes with the greatest weekly mileage were also the athletes who tended to resume normal menses. However the spontaneous resumption group tended to have decreased their mileage over time to a somewhat larger degree than the group that remained irregular. It would be of interest to

study the effects of changes in level of physical activity in a larger study population.

Finally, it would be expected that an increase in body weight would reflect a greater energy reserve and be associated with a resumption of menstrual function. In this analysis, weight and percent body fat and increases in weight and fat did not differ significantly between those who did and those who did not resume normal menstrual function. However, as seen in project (2), increases in weight and fat tend to result in subsequent caloric restriction and increases in the eating disorder inventory score among oligo/amenorrheic women. With the relatively small number of women included in project (4), it is difficult to sort out the role of these various interrelated factors.

Limitations

A major limitation is of course the small sample size. Reliance on self-report of most variables and lack of hormone measurements are other limitations.

Table 1. Characteristics of Subsample Used in These Analyses by Menstrual Status at Last Follow-up: Mean (and Standard Deviation)

Characteristics	Periods Remained Irregular N=11 Mean (SD)	Regained Periods Spontaneously N=14 Mean (SD)	Mean of 2 groups N=25 Mean (SD)
Age in years	23.0 (2.7)	21.3 (3.0)	22.0 (2.9)
Weight in pounds	127.3 (12.9)	130.2 (12.1)	128.9 (12.3)
Height in inches	66.2 (2.1)	65.7 (2.5)	65.9 (2.3)
Body mass index	20.5 (1.5)	21.2 (1.3)	20.9 (1.4)
Percent body fat	23.9 (6.5)	22.8 (5.1)	23.3 (5.7)
Age at menarche in years	13.6 (1.5)	13.9 (1.5)	13.7 (1.5)
Average number of periods per year since menarche	6.3 (1.7)	6.3 (2.6)	6.3 (2.2)
Number of menses in past year	6.0 (2.9)	5.1 (3.2)	5.5 (3.0)
Age in years at which started training	14.5 (3.6)	13.4 (3.8)	13.8 (3.7)
Number of competitive seasons run	10.2 (6.4)	12.6 (6.0)	11.5 (6.2)

Table 2. Mean (and standard deviation) and changes in mean (and standard deviation) for selected variables by menstrual status at last follow-up

Variable	Remained Irregular Group N=11*	Regained Spontaneously Group N=14*	p-value
Score on anorexia subscale of Eating Disorder Inventory:			
At baseline	6.6 (5.9)	9.2 (7.3)	.35
At follow-up 1	7.7 (6.1)	7.1 (6.5)	.84
At follow-up 2	5.6 (5.1)	8.6 (7.6)	.36
Change from baseline to 1	1.1 (2.1)	-1.0 (2.4)	.05
Change from baseline to 3	0.8 (6.8)	-0.5 (2.7)	.60
Total Eating Disorder Inventory score			
At baseline	14.1 (10.7)	20.3 (14.1)	.24
At follow-up 1	16.2 (11.0)	18.8 (16.4)	.70
At follow-up 2	13.0 (12.2)	18.5 (16.1)	.43
Change from baseline to 1	2.2 (6.3)	-.3 (7.5)	.43
Change from baseline to 3	1.0 (15.1)	-1.3 (8.8)	.69
Daily kilocalories consumed			
At baseline	2135 (608)	2780 (1025)	.08
At follow-up 1	1643 (448)	2198 (1312)	.24

At follow-up 2	1637 (417)	2336 (1056)	.12
Change baseline to 1	-339 (489)	-437 (606)	.70
Change from baseline to 2	-324 (328)	-529 (681)	.48
Percentage of daily kilocalories from fat			
At baseline	17.8 (5.7)	15.1 (4.7)	.22
At follow-up 1	18.4 (7.0)	19.5 (6.7)	.72
At follow-up 2	18.6 (4.0)	19.9 (7.3)	.66
Change from baseline to 1	0.1 (3.1)	4.5 (5.5)	.05
Change from baseline to 2	1.1 (2.8)	5.2 (6.2)	.12
Miles run per week			
At baseline	31.0 (9.9)	42.1 (16.4)	.06
At follow-up 1	27.2 (10.4)	38.3 (15.1)	.07
At follow-up 2	27.1 (13.4)	34.8 (17.4)	.31
Change from baseline to 1	-3.2 (12.6)	-4.2 (10.2)	.84
Change from baseline to 2	-2.2 (12.5)	-7.1 (13.6)	.43
Weight in pounds			
At baseline	127.3 (12.9)	130.2 (12.1)	.57
At follow-up 1	125.6 (12.9)	129.6 (14.2)	.50
At follow-up 2	126.6 (17.4)	135.0 (12.1)	.25
Change from baseline to 1	-.6 (3.9)	-1.1 (6.2)	.85
Change from baseline to 2	-2.1 (15.5)	5.1 (8.3)	.23
Percent body fat			

At baseline	24.0 (6.5)	22.8 (5.1)	.62
At follow-up 1	23.2 (6.6)	23.4 (4.4)	.93
At follow-up 2	23.4 (10.4)	22.0 (6.3)	.75
Change from baseline to 1	-.6 (1.3)	-.2 (3.8)	.79
Change from baseline to 2	-1.8 (7.2)	1.0 (4.5)	.35
Energy balance: Daily kilocalories consumed minus daily kilocalories expended in running			
At baseline	1781 (606)	2288 (1136)	.19
At follow-up 1	1305 (311)	1995 (1523)	.26
At follow-up 2	1331 (328)	1652 (818)	.35
Change from baseline to 1	-276 (622)	-263 (617)	.97
Change from baseline to 2	-308 (388)	-547 (658)	.42

*N=25 for all variables at baseline. At follow-up visits 1 and 2, N<25 because of missing data.

(7) KEY RESEARCH ACCOMPLISHMENTS:

Results related to bone health:

- A two-year randomized trial of the effect of oral contraceptives on bone mass and stress fracture occurrence in young female distance runners was completed.
- Randomization to oral contraceptives was unrelated to changes in bone mineral density or bone mineral content in either oligo/amenorrheic or eumenorrhic runners.
- When actual oral contraceptive use was considered (rather than the group to which women were randomly assigned), oligo/amenorrheic runners who used oral contraceptives gained about 1% spine bone mineral density and whole-body bone mineral content, an amount similar to the gain in those who regained periods spontaneously and significantly greater than those who remained oligo/amenorrheic.
- Oral contraceptives may protect against stress fractures, but results are not definitive.
- Milk consumption was associated with a decreased risk for stress fracture and longitudinal increases in hip bone mineral density and whole-body bone mineral content.
- Risk factors for stress fracture in this study were previous stress fractures, lower bone mass, younger chronologic age, lower dietary calcium intake, and younger age at menarche, and possibly a history of irregular menstrual periods.
- Training-related factors were not related to stress fracture risk.

- Gains in weight, fat mass, percent body fat, and lean mass were associated with increases in bone mineral density in the spine and hip, but only among those who were initially oligo/amenorrheic.

Secondary analyses: results related to weight and body composition

- Oral contraceptives did not cause weight or fat gain.
- Oral contraceptives may be associated with lean mass gain among eumenorrheic, but not among amenorrheic and oligomenorrheic runners.
- Running more miles per week was associated with reduction in weight and fat mass, but not lean mass.
- Weekly duration of weight lifting was associated with increases in lean mass, but not weight or fat mass.
- Runners who gained weight and fat mass subsequently tended to restrict their total caloric intake.
- Oligo/amenorrheic runners who gained weight and fat mass subsequently tended to show increases in their eating disorder score.

Secondary analysis: factors possibly associated with return to regular menses among those amenorrheic or oligomenorrheic at baseline:

- Return to regular menses among those with irregular menses may be associated with more daily calories consumed, more fat consumption, a lower tendency towards eating disorders, and a greater number of miles run per week early in the study but a slightly greater decrease in weekly miles run over time..

(8) REPORTABLE OUTCOMES

Cobb KL, Bachrach, LK, Greendale G, Marcus R, Neer R, Nieves J, Sowers MF, Brown BW, Gopalakrishnan G, Luetters C, Tanner HK, Ward B, Kelsey JL. Disordered eating, menstrual irregularity, and bone mineral density in female runners. *Med Sci Sports Exerc*, 35:711-9, 2003.

Cobb KL, Bachrach LK, Sowers M, Nieves J, Greendale, GA, Kent KK, Brown BW Jr., Pettit K, Harper, DM, Kelsey JL. The effect of oral contraceptives on bone mass and stress fractures in female runners. *Med Sci Sports Exerc*, in press.

Kelsey JL, Bachrach LK, Procter-Gray E, Nieves J, Greendale GA, Sowers M, Brown BW, Jr., Matheson KA, Crawford SL, Cobb, KL. Risk factors for stress fracture among young female cross-country runners. *Med Sci Sports Exerc*, in press.

Procter-Gray, E et al. The effect of oral contraceptives on body weight and body composition in young female runners. To be submitted to *Med Sci Sports Exerc*.

(9) LIST OF PERSONNEL RECEIVING PAY FROM RESEARCH EFFORT

Stanford: Jennifer Kelsey, Laura Bachrach, W. Byron Brown, Jr., Robert Marcus, Bridget Ward, Katrina Mogielnicki, Kyla Kent, Caroline Annis, Crystal Luetters, Kimberly Matheson, Elizabeth Procter-Gray, Kathryn Melsop, Mila Prill, Heather Tanner, Ilse Larson, Meredith Curtis, Audrey Ahuero, Anna Minta, Kate Petit, Elizabeth Levey, Eleni Greenwood, Kelly Shouey, Kari Meyer.

Helen Hayes Hospital: None.

Massachusetts General Hospital: Robert Neer.

University of California at Los Angeles: Gail Greendale, Anna Chirra, Crystal Luetters, Valerie Braun, Anna McDivit.

University of Michigan: MaryFran Sowers, Jane Seaver, Erin Dotson, Priscilla Marbury.

(10) CONCLUSIONS (adapted from manuscript Abstracts)

In order to gain bone mass, oligo/amenorrheic athletes with low bone mass should be advised to gain weight, increase dietary calcium, and take steps to resume normal menses; they may benefit from oral contraceptives, but our results are not conclusive. Oral contraceptives may reduce the risk for stress fracture in female runners, but our data are again not conclusive. The oral contraceptive used in this study, Lovral, was not associated with weight or fat mass gain. Further study is needed to evaluate our finding that use of this oral contraceptive was associated with lean mass gain in eumenorrheic women. The results of this and other studies indicate that risk factors for stress fracture among young female runners include one or more previous stress fractures, lower bone mass, and, although not statistically significant in the present study, menstrual irregularity. More study is needed of the associations between risk for stress fracture according to age, calcium intake, and age at menarche. Because of difficulty in recruitment and because many young women have reason to switch onto or off oral contraceptives during a trial, it will be difficult to conduct a randomized trial that definitely answers the question of whether use of oral contraceptives protects against loss of bone mass and reduces the risk for stress fractures in young female distance runners.

(11) REFERENCES: None

(12) APPENDICES: See attached publication, two manuscripts in press, and one manuscript about to be submitted.

Disordered Eating, Menstrual Irregularity, and Bone Mineral Density in Female Runners

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¹Division of Epidemiology, Department of Health Research and Policy, ²Department of Pediatrics, Stanford University School of Medicine, Stanford, CA; ³UCLA School of Medicine, Los Angeles, CA; ⁴Veterans Affairs Medical Center and Stanford Medical School, Palo Alto, CA; ⁵Massachusetts General Hospital and Harvard Medical School, Boston, MA; ⁶Clinical Research Center, Helen Hayes Hospital and Columbia University, New York, NY; and ⁷Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI

ABSTRACT

COBB, K. L., L. K. BACHRACH, G. GREENDALE, R. MARCUS, R. M. NEER, J. NIEVES, M. F. SOWERS, B. W. BROWN, JR., G. GOPALAKRISHNAN, C. LUETTERS, H. K. TANNER, B. WARD, and J. L. KELSEY. Disordered Eating, Menstrual Irregularity, and Bone Mineral Density in Female Runners. *Med. Sci. Sports Exerc.*, Vol. 35, No. 5, pp. 711–719, 2003. Purpose: To examine the relationships between disordered eating, menstrual irregularity, and low bone mineral density (BMD) in young female runners. Methods: Subjects were 91 competitive female distance runners aged 18–26 yr. Disordered eating was measured by the Eating Disorder Inventory (EDI). Menstrual irregularity was defined as oligo/amenorrhea (0–9 menses per year). BMD was measured by dual x-ray absorptiometry. Results: An elevated score on the EDI (highest quartile) was associated with oligo/amenorrhea, after adjusting for percent body fat, age, miles run per week, age at menarche, and dietary fat. (OR [95% CI]: 4.6 [1.1–18.6]). Oligo/amenorrheic runners had lower BMD than eumenorrheic runners at the spine (−5%), hip (−6%), and whole body (−3%), even after accounting for weight, percent body fat, EDI score, and age at menarche. Eumenorrheic runners with elevated EDI scores had lower BMD than eumenorrheic runners with normal EDI scores at the spine (−11%), with trends at the hip (−5%) and whole body (−5%), after adjusting for differences in weight and percent body fat. Runners with both an elevated EDI score and oligo/amenorrhea had no further reduction in BMD than runners with only one of these risk factors. Conclusion: In young competitive female distance runners, (i) disordered eating is strongly related to menstrual irregularity, (ii) menstrual irregularity is associated with low BMD, and (iii) disordered eating is associated with low BMD in the absence of menstrual irregularity. Key Words: FEMALE ATHLETES, LONG DISTANCE, OSTEOPENIA, OSTEOFOSIS, AMENORRHEA, OLIGOMENORRHEA, EATING ATTITUDES, EATING DISORDER INVENTORY, FEMALE ATHLETE TRIAD

The “female athlete triad” (33) is the combination of disordered eating, menstrual irregularity, and osteoporosis/osteopenia seen in young female athletes. Disordered eating, which affects as many as two thirds of young female athletes (33), consists of restrictive eating behaviors that do not necessarily reach the level of a clinical eating disorder (2). Women athletes with disordered eating

may limit their caloric and/or fat intakes but maintain high training levels, often resulting in a state of chronic energy deficit. Among other adverse consequences, energy imbalance has been linked to depressed estrogen levels, metabolic disturbances, and amenorrhea or oligomenorrhea (2,7,26,34,49,50). Amenorrheic/oligomenorrheic athletes on average have lower bone mineral density (BMD) than eumenorrheic controls (6,7,9,20,22–24,26,28,29,32,34,37–39,46,48,49). This bone deficit may be related to an increased incidence of stress fractures (1,10,30) and may be only partially reversible (16,18,21) putting women at risk for life-long health consequences.

The existence of the female athlete triad is implicit in studies that established a relationship between eating behaviors and menstrual irregularity (2,7,26,34,39,49,50) and those that established a relationship between menstrual irregularity and low BMD (6,7,9,20,22–24,26,28,29,32,34,37–39,45,47,48). How-

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ever, few studies have actually measured menstruation, diet, and BMD simultaneously (7,23,26,34,39), and these studies were conducted, largely, before the female athlete triad was recognized as a distinct syndrome. Therefore, the female athlete triad has yet to be explored as a triad, and the complex relationships among all three components have yet to be established.

In this article, we examine eating attitudes and patterns, menstrual status, and BMD in a group of 91 competitive female distance runners, using data collected at the baseline examination of a randomized controlled trial. We examine the etiology of menstrual irregularity in this population, specifically as it relates to diet and eating behaviors. We address the question of whether low body weight can explain the differences in BMD between eumenorrheic and oligo/amenorrheic athletes, as several researchers have suggested (6,32,45,48) or if menstrual irregularity is associated with BMD independently of low weight. Finally, we examine the relationship between disordered eating and BMD independent from menstrual irregularity, a link that has not been well studied in female athletes.

MATERIALS AND METHODS

We analyzed the baseline cross-sectional data from 91 competitive female long-distance runners, aged 18–25 yr, who enrolled in a randomized controlled trial to examine the effect of oral contraceptives on BMD in female runners.

Subjects. Women were recruited from intercollegiate cross-country teams, postcollegiate running clubs, and road-race participants in the geographic areas of Palo Alto, CA; Los Angeles, CA; Ann Arbor, MI; West Haverstraw, NY; and Boston, MA. To be eligible, women had to run at least 40 miles·wk⁻¹ during peak training times, and they had to compete in running races. Additionally, because the women were recruited as part of a randomized trial of oral contraceptives, they could not have used oral contraceptives or other hormonal contraception within 6 months before entering the study; they had to be willing to be randomized to take oral contraceptives or not to take them; and they could have no medical contraindications to oral contraceptive use. All women were required to visit a study physician or student health service staff member before enrollment in the study. Details of the study and testing procedures were explained to each subject, and a written, informed consent was obtained. The experimental protocol was approved by the Institutional Review Boards of Stanford University, the University of California, Los Angeles, the University of Michigan, the Helen Hayes Hospital, and Massachusetts General Hospital.

Questionnaire. A self-administered questionnaire was used to assess training regimen and menstrual history. Women were asked to record the number of miles they ran per week during each competitive season (fall cross-country, winter track, spring track) and the off-season (summer) in the past 12 months. From this information, an average number of miles run per week was calculated for the year before study enrollment.

Women reported the number of menses in the previous 12 months and were classified, accordingly, as eumenorrheic (10 or more cycles in the past year), oligomenorrheic (4–9 menstrual cycles per year), or amenorrheic (fewer than 4 cycles in the past year) (40). Menstrual irregularity has been defined as 0–9 menses per year in previous studies of young women runners (1,28,30,39), and we used that definition here. Both oligomenorrheic and amenorrheic athletes have previously been found to have lower serum estradiol concentrations (24,39,42) and to have lower BMD than eumenorrheic athletes (6,24,38,39,42). In our study population, amenorrheic and oligomenorrheic athletes were similar in BMD, EDI scores, and past menstrual irregularity, justifying their combination into a single group. Women recorded their age at menarche and indicated whether they had had 0, 1–3, 4–9, or 10–13 menses during each year after menarche. Total lifetime menses was calculated using the midpoint of each of these categories. The total number of past years of amenorrhea was calculated by summing the number of years for which women checked “0” or “1–3” periods, excluding the year of menarche and the current year. Past oligomenorrheic years were calculated similarly, except using the category “4–9” periods.

Diet and eating behaviors. An expanded version of the 97-item National Cancer Institute Health Habits and History food frequency questionnaire (4) was used to estimate usual nutrient intake during the prior 6 months. We modified the questionnaire to accommodate the special diets of college-aged female athletes by adding low-fat and non-fat versions of certain foods, vegetarian and vegan foods, ethnic foods, and sports nutrition products (such as Gatorade and Power Bars). The nutrient contents of the added foods were obtained from the U.S. Department of Agriculture Nutrient Database for Standard Reference, release 14 (<http://www.nal.usda.gov/fnic/foodcomp>), and from food labels. Total intakes of energy, protein, fat, carbohydrates, calcium, phosphorous, iron, fiber, and vitamin C were calculated.

Three subscales (drive for thinness, bulimic tendencies, and body dissatisfaction) of the Eating Disorder Inventory (EDI) were used to screen for subclinical eating disorders (2,12,13). Athletes with subclinical eating disorders have previously been shown to have significantly elevated scores on these three subscales of the EDI (2,11,13,35). Responses on each EDI subscale were scored separately and also totaled.

Physical and bone measurements. At each of the five clinical assessment sites, height and weight were measured using standard stadiometers and balance-beam scales, respectively. Body mass index was calculated as kilograms per square meter.

BMD ($\text{g}\cdot\text{cm}^{-2}$) at the left proximal femur, spine, and whole body, and body composition (lean body mass and fat mass) were measured by dual energy x-ray absorptiometry (DXA; QDR 4500A, Hologic). The coefficient of variation for these machines is less than 1.0% for all bone sites (<http://www.hologic.com/prod-bd/pdf/spec-4500.pdf>). Machines were cross-calibrated using a circulating Hologic anthropomorphic spine phantom. Each site maintained a

TABLE 1. Mean \pm 1 SEM for selected physical and reproductive characteristics, and training variables, by menstrual group.

Characteristic	Menstrual Group	
	Eumenorrheic (N = 58)	Oligo/Amenorrheic* (N = 33)
Age (yr)	21.7 \pm 0.3	21.8 \pm 0.5
Weight (lb)	129.1 \pm 1.9	128.1 \pm 2.7
Height (inches)	65.1 \pm 0.3	65.4 \pm 0.5
BMI ($\text{kg}\cdot\text{m}^{-2}$)	21.5 \pm 0.2	21.1 \pm 0.3
Body fat (%)	23.9 \pm 0.6	22.7 \pm 1.0
Menses in past year (no. cycles)	11.5 \pm 0.1	5.0 \pm 0.5†
Menarche (age in yr)	12.6 \pm 0.2	13.8 \pm 0.2‡
Total lifetime menstrual periods (no. cycles)	89.5 \pm 4.4	49.5 \pm 4.0‡
Started running (age in yr)	14.5 \pm 0.5	14.7 \pm 0.7
Amount of running (miles·wk ⁻¹ in past 12 months)	33.0 \pm 1.2	39.0 \pm 2.2§

* Oligoamenorrhea was defined as 0–9 menses over the past 12 months.

† P < 0.0001, Wilcoxon signed rank test.

‡ P < 0.0001, t-test.

§ P < 0.05, t-test.

standard quality assurance program. All women were asked to refrain from heavy physical activity 24 h before screening to minimize the effect of fluctuations in hydration status on body composition measurements.

Statistical analyses. Statistical analyses were performed using the SAS statistical package, version 6.12 (SAS Institute, Cary, NC). Means were compared between groups using t-tests for normally distributed variables and the Wilcoxon signed rank test for nonnormally distributed variables. Tukey's multiple comparisons test was used to compare mean BMD across more than two groups. ANCOVA was used to control for age, weight, and body composition.

The relationships between oligo/amenorrhea and training, diet, and physical characteristics were assessed by multiple logistic regression. Multiple linear regression was used to examine the effects of menstrual group and EDI score on BMD when considering EDI score as a continuous variable.

RESULTS

Thirty-six percent of the study sample met criteria for abnormal menses; 26% were oligomenorrheic and 10% were amenorrheic during the past year. Oligo/amenorrheic

women were similar to eumenorrheic women in age, weight, height, and body composition (Table 1). The oligo/amenorrheic women had menarche a mean of 1.2 yr later and had an average of 45% fewer menstrual periods in their lifetime than eumenorrheic women. They also ran an average of 18% more miles per week than eumenorrheic women.

Disordered eating and menstrual irregularity. The women were divided into two groups (normal EDI/elevated EDI) by their total scores on three subscales of the EDI. Women in the highest quartile of total EDI were classified as having elevated EDI scores compared with women in the lowest three quartiles. Women in the elevated EDI group had EDI values comparable to those previously published for patients with anorexia nervosa (12) on the drive for thinness and body dissatisfaction subscales (Table 2). Athletes with elevated EDI scores reported 19% lower daily caloric intakes compared with women with normal EDI scores (Table 2) and reported that they obtained 25% fewer of those calories from fat. The groups were similar in consumption of other nutrients. Although the elevated EDI group had a somewhat lower daily calcium intake, this was proportional to their lower energy intake. Both groups, on average, consumed

TABLE 2. Mean \pm 1 SEM for selected diet and nutrition characteristics by eating disorder inventory (EDI) group and, for comparison, a previously published anorectic group.

Characteristic	EDI Group (This Study)		
	Normal EDI (N = 67)	Elevated EDI* (N = 23)	Anorectics (Previously Published)† (N = 155)
EDI scores*			
Drive for thinness subscale (0–21)	1.6 \pm 0.3	16.3 \pm 0.8‡	13.8 \pm 0.5
Bulimia subscale (0–21)	0.8 \pm 0.2	3.2 \pm 0.7‡	8.1 \pm 0.5
Body dissatisfaction subscale (0–27)	3.6 \pm 0.5	16.0 \pm 1.2‡	15.5 \pm 0.6
Total (0–69)	6.0 \pm 0.8	35.6 \pm 1.8‡	37.4 \pm 0.9
Daily nutrient intake			
Calories (kcal·d ⁻¹)	2346 \pm 112	1904 \pm 148§	
Fat (% of total calories)	18.7 \pm 0.8	14.0 \pm 1.0†	
Protein (% of total calories)	16.4 \pm 0.3	16.0 \pm 0.8	
Calcium (mg)	1467 \pm 96	1300 \pm 147	
Fiber (g)	30.6 \pm 2.5	26.4 \pm 2.2	
Vitamin C (mg)	291 \pm 23	247 \pm 23	
Iron (mg)	23.6 \pm 2.3	20.0 \pm 1.9	

* EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead (12). Elevated scores are defined as the highest quartile (≥ 23). One subject is missing EDI scores; therefore, she was removed from all analyses involving EDI.

† Average scores for anorexia nervosa patients as published by Garner and Olmstead (12).

‡ Elevated EDI group vs normal EDI group, P < 0.0001, Wilcoxon signed rank test.

§ Elevated EDI group vs normal EDI group, P < 0.05, t-test.

† Elevated EDI group vs normal EDI group, P < 0.01, t-test.

TABLE 3. Mean \pm 1 SEM for selected diet and nutrition characteristics by menstrual group.

Characteristic	Menstrual Group	
	Eumenorrheic (N = 58)	Oligo/Amenorrheic (N = 33)
EDI scores*		
Drive for thinness subscale (0–21)	3.3 \pm 0.7	9.3 \pm 1.4†
Bulimia subscale (0–21)	0.9 \pm 0.2	2.3 \pm 0.5‡
Body dissatisfaction subscale (0–27)	5.4 \pm 0.8	9.3 \pm 1.5‡
total (0–69)	9.6 \pm 1.5	20.0 \pm 3.0†
Daily nutrient intake		
Calories (kcal·d ⁻¹)	2241 \pm 121	2219 \pm 147
Fat (% of total calories)	18.7 \pm 0.9	15.3 \pm 1.0§
Protein (% of total calories)	16.3 \pm 0.4	16.3 \pm 0.5
Calcium (mg)	1418 \pm 106	1437 \pm 123
Fiber (g)	28.1 \pm 2.2	32.0 \pm 3.7
Vitamin C (mg)	283 \pm 23	274 \pm 28
Iron (mg)	22.2 \pm 2.6	23.6 \pm 2.1

* EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead (12).

† Oligoamenorrheic vs eumenorrheic, $P < 0.005$, Wilcoxon signed rank test.

‡ Eumenorrheic vs oligo/amenorrheic, $P < 0.05$, t-test.

§ Eumenorrheic vs oligo/amenorrheic, $P < 0.05$, t-test.

greater than 1200 mg of calcium per day, which is the U.S. recommended daily allowance for this age group.

Of 23 women with elevated EDI scores, 65% had oligo/amenorrhea, whereas only 25% of 67 women with normal EDI scores did. Though each of the three EDI subscales scores was higher in the oligo/amenorrheic group, the drive for thinness EDI subscale had the strongest association with oligo/amenorrhea (Table 3). Oligo/amenorrheic athletes and eumenorrheic athletes were similar in daily nutrient profiles, though oligo/amenorrheic athletes reported a lower percentage of their calories from fat (Table 3).

Table 4 shows odds ratios for several factors associated with oligo/amenorrhea. Being in the top quartile of EDI score conferred fourfold-increased odds of oligo/amenorrhea. Every 1-yr increase in age at menarche was associated with a more than twofold increase in the odds of oligo/amenorrhea. Odds of oligo/amenorrhea were also increased with greater miles run per week and were decreased with a higher percent body fat and with a higher percent fat intake, but the confidence intervals for these associations included one. Total energy intake was not associated with menstrual disturbances.

EDI score and percent fat intake were modestly negatively correlated (Spearman rank correlation coefficient: $r = -0.34$) and reduced fat intake may lie in the causal pathway between elevated EDI and oligo/amenorrhea. If dietary fat is removed from the logistic regression model, the OR for elevated EDI score increases from 4.6 to 6.7 (1.8, 25.6), suggesting that low fat intake accounts for some of the association between elevated EDI and menstrual irregularity.

TABLE 4. Odds ratios (and 95% confidence intervals) for the association between selected characteristics and oligomenorrheal/amenorrhea.*

Characteristic	Odds Ratios (95% CI)
Elevated EDI score (≥ 23 vs < 23)	4.56 (1.12, 18.61)
Menarche (each 1 yr later)	2.45 (1.46, 4.11)
Miles per week (every 10 miles)	1.64 (0.96, 2.79)
Dietary fat (every 5% of total calories)	0.61 (0.36, 1.03)
Body fat (every 5%)	0.56 (0.30, 1.07)

* Adjusted for age and each of the other variables in the table by multiple logistic regression.

TABLE 5. Observed and adjusted* spine, hip, and whole body bone mineral density (BMD, g·cm⁻² \pm 1 SEM), by menstrual group.

Characteristic	Menstrual Group	
	Eumenorrheic (N = 58)	Oligo/Amenorrheic (N = 33)
Spine BMD		
Observed	1.01 \pm 0.013	0.94 \pm 0.018‡
Adjusted*	1.00 \pm 0.013	0.95 \pm 0.019§
Total hip BMD		
Observed	1.00 \pm 0.015	0.95 \pm 0.020§
Adjusted*	1.00 \pm 0.014	0.94 \pm 0.020§
Whole body BMD		
Observed	1.12 \pm 0.011	1.08 \pm 0.015§
Adjusted*	1.11 \pm 0.010	1.08 \pm 0.015

* Adjusted for age, body weight, percent body fat, EDI score, and age at menarche by analysis of covariance.

† Oligo/amenorrhea was defined as 0–9 menses over the past 12 months.

‡ Eumenorrheic vs oligo/amenorrheic, $P < 0.005$, t-test.

§ Eumenorrheic vs oligo/amenorrheic, $P < 0.05$, t-test.

ity. EDI score was not correlated with miles run per week (Spearman rank correlation coefficient: $r = 0.01$), so increased training, though related to oligo/amenorrhea, does not mediate the relationship between elevated EDI scores and oligo/amenorrhea.

Menstrual irregularity and BMD. BMD was 5%, 6%, and 3% lower at the lumbar spine, total hip, and whole body, respectively, in oligo/amenorrheic women compared with eumenorrheic women after adjustment for weight, percent body fat, EDI score, and age at menarche (Table 5). Adjusted and unadjusted BMD values were similar (Table 5); thus, although weight was strongly correlated with BMD at all skeletal sites (the Pearson correlation coefficients were whole body: $r = 0.43$; hip: $r = 0.40$; and spine: $r = 0.38$), lower weight did not account for the association between menstrual irregularity and low BMD in this study population.

Disordered eating and BMD. There were no differences in BMD between women with elevated EDI scores and women with normal EDI scores before adjusting for body size. However, women with elevated EDI scores were heavier (138.5 ± 3.2 lb) and had a higher percent body fat ($25.7 \pm 1.1\%$) than those with normal EDI scores (125.8 ± 1.7 lb; $22.8 \pm 0.6\%$). Based on multiple linear regression, we would expect the women with elevated EDI to have $0.038 \text{ g} \cdot \text{cm}^{-2}$ greater BMD at the spine and hip and $0.028 \text{ g} \cdot \text{cm}^{-2}$ greater BMD at the whole body due to their higher weight (correcting for their higher percent body fat). Once we adjusted for body weight and composition, women with elevated EDI scores had significantly lower BMD compared with women with normal EDI scores at the spine (-6%), with trends at the hip (-3%) and whole body (-4%).

Menstrual status modified the effect of EDI score on adjusted BMD (Table 6). Among eumenorrheic women, those with elevated EDI scores had significantly lower spine BMD and nonsignificant trends for lower hip and whole body BMD compared with women with normal EDI scores (Table 6). These differences were not attributable to past menstrual history, which was similar in the two groups. Among oligo/amenorrheic women, however, there were no trends for lower BMD among women with elevated EDI compared with women with normal EDI.

TABLE 6. Observed and adjusted* spine, hip, and whole body bone mineral density ($\text{g}\cdot\text{cm}^{-2} \pm 1 \text{ SEM}$) by combined menstrual and eating disorder inventory (EDI) groups.

	Group			
	1	2	3	4
EDI score group†	Normal	Normal	Elevated	Elevated
Menstruation	Eumenorrhea	Oligo/amenorrhea‡	Eumenorrhea	Oligo/amenorrhea
N	50	17	8	15
Mean weight (lb \pm SE)	126.3 \pm 1.8	123.5 \pm 4.3	146.4 \pm 5.7	133.4 \pm 3.2
Spine BMD ($\text{g}\cdot\text{cm}^{-2} \pm \text{SE}$)				
Observed	1.02 \pm 0.015	0.90 \pm 0.024§	0.97 \pm 0.027	0.97 \pm 0.025
Adjusted*	1.02 \pm 0.014	0.93 \pm 0.024§	0.91 \pm 0.036	0.96 \pm 0.025**
Total hip BMD ($\text{g}\cdot\text{cm}^{-2} \pm \text{SE}$)				
Observed	1.00 \pm 0.016	0.91 \pm 0.038	1.00 \pm 0.023	0.98 \pm 0.032
Adjusted*	1.01 \pm 0.015	0.93 \pm 0.027	0.96 \pm 0.040	0.96 \pm 0.027
Whole-body BMD ($\text{g}\cdot\text{cm}^{-2} \pm \text{SE}$)				
Observed	1.12 \pm 0.013	1.07 \pm 0.018	1.12 \pm 0.029	1.09 \pm 0.016
Adjusted*	1.13 \pm 0.010	1.08 \pm 0.019	1.07 \pm 0.028	1.08 \pm 0.020

* Adjusted for body weight, percent body fat, age, and age at menarche by analysis of covariance.

† EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead (12). Elevated scores are defined as the highest quartile (≥ 23). One subject is missing EDI score; therefore, she was removed from all analyses involving EDI.

‡ Oligo/amenorrhea was defined as ≥ 6 months over the past 12 months.

§ Group 1 vs group 2, $P < 0.005$, Tukey's test for comparing multiple group means.

|| Group 1 vs group 2; group 1 vs group 3, $P < 0.05$, Tukey's.

** Group 1 vs group 4, $P < 0.10$, Tukey's.

Multiple linear regression analysis confirmed the significant interactions between menstrual irregularity and total EDI score (0–69) on BMD at all skeletal sites (Fig. 1). Among eumenorrheic runners, EDI score is inversely related to BMD. However, among oligo/amenorrheic women, BMD is not related to EDI score. Similarly, among women with low EDI scores, oligo/amenorrheic women had lower BMD than eumenorrheic women, but, among women with high EDI scores, menstrual irregularity was not related to BMD.

DISCUSSION

This study confirms the existence and significance of the "female athlete triad," a syndrome composed of three interrelated conditions: disordered eating, menstrual irregularity, and osteopenia/osteoporosis (33). (i) We confirm that disordered eating in female runners is correlated with oligo/amenorrhea; (ii) we demonstrate that the association be-

tween oligo/amenorrhea and low BMD in female runners is independent of body weight and body composition; and (iii) we provide novel evidence that disordered eating is associated with low BMD in eumenorrheic women runners.

The women in our study who were in the highest quartile of total EDI score had similar values on two EDI subscales to patients with diagnosed anorexia nervosa (12); they also had similar or slightly higher EDI scores than women athletes with established subclinical eating disorders (2,11,13,35). The EDI measures only attitudes about food and body size. However, we verified that elevated scores on the EDI translated to actual eating practices; women with elevated EDI scores reported lower total energy intakes (by approximately 19% d^{-1}) and lower percent fat intakes (by approximately 25% d^{-1}) than women with normal EDI scores. None of the 91 women in our study indicated that she was dieting to lose weight (data not shown), suggesting that this observed dietary restriction

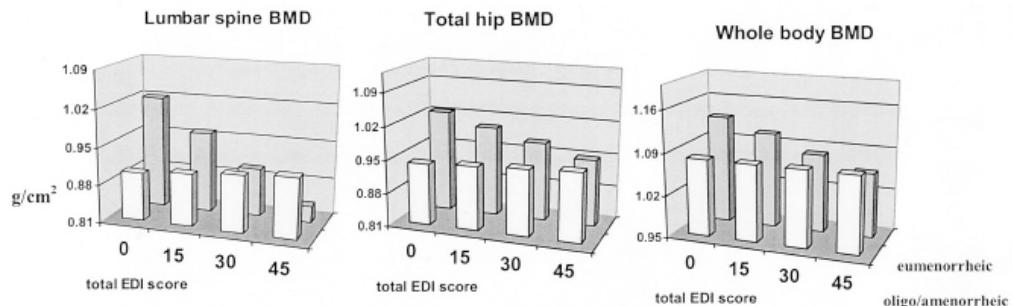


FIGURE 1—Mean BMD ($\text{g}\cdot\text{cm}^{-2}$) at the spine, hip, and whole body by menstrual status and varying levels of total EDI score (from multiple linear regression). The means are based on the following multiple linear regression results (adjusted for age, weight, and percent body fat). Regression coefficients (standard error)—menstrual status $-0.12 (0.33)$, EDI score (0–69) $-0.004 (0.001)$, interaction: menstrual status \times EDI score $+0.0043 (0.002)$; menstrual status $-0.089 (0.036)$, EDI score (0–69) $-0.002 (0.001)$, interaction: menstrual status \times EDI score $+0.002 (0.002)$; menstrual status $-0.060 (0.025)$, EDI score (0–69) $-0.002 (0.001)$, interaction: menstrual status \times EDI score $+0.002 (0.001)$. (Menstrual status equals 1 if the woman is oligo/amenorrheic and 0 if the woman is eumenorrheic.)

represents long-term, chronic restriction, rather than temporary attempts to lose weight.

Women with elevated EDI scores had a fourfold increase in risk for oligo/amenorrhea, when controlling for other factors. Chronic energy deficit has previously been implicated in the etiology of athletic amenorrhea (2,7,8,19,25,26,31,34,44,46,49,50). Menstruation requires a small amount of energy, and halting menstruation may be an adaptive energy-conservation mechanism. In our study, the caloric restriction of the elevated EDI group did not appear to explain their excess oligo/amenorrhea. Rather, our data suggest that the development of oligo/amenorrhea in these women may have been mediated in part by a reduction of dietary fat intake. Though dietary fat, independent of total energy intake, has previously been shown to influence the menstrual cycle in nonathletic women (15,27), this association has not previously been demonstrated in female athletes and needs verification in further studies. We speculate that women with disordered eating may have more aberrant patterns of eating, such as binging and fasting cycles; although total energy intake may not be altered, these patterns have potential to alter metabolic pathways, hormone levels, and, ultimately, menstruation (3,5,14).

We found that oligo/amenorrheic runners ran more miles per week than eumenorrheic runners. Therefore, although energy intake was not associated with menstrual irregularity, oligo/amenorrheic runners may have had greater energy imbalance due to a higher energy expenditure. Energy imbalance may cause hypothalamic dysfunction, which disrupts both menses and bone remodeling (50).

We confirm previous research that shows that delayed menarche is a strong predictor of later menstrual irregularity (6,9,29,34). Delayed menarche was correlated with menstrual irregularity in both women who initiated training before menarche ($N = 22$) and women who started training after menarche ($N = 69$); thus, prior training does not explain the delay in menarche in the oligo/amenorrheic runners. This finding suggests that some women may be predisposed to menstrual irregularity, which may account for the existence of a subset of women with low total EDI scores (6.8 ± 1.8) and putatively sufficient caloric intake (2443 ± 210) who still lost their periods. Alternatively, disordered eating patterns may have developed premenarche and pretraining in certain women which contributed to a delay in the onset of menarche and has subsequently continued to disrupt the menses. Our data were insufficient to evaluate this hypothesis.

We confirm numerous reports of reduced BMD in oligo/amenorrheic female athletes, with the largest and most consistent effects having been demonstrated at the lumbar spine. The BMD differences between oligo/amenorrheic and eumenorrheic women that we observed were not attributable to differences in body weight, body composition, or EDI score. The magnitude of the difference was important; 6% of the oligo/amenorrheic young women had spine BMD values that would be considered osteoporotic, that is, a BMD value less than 2.5 SD below young adult BMD (17) ($<0.772 \text{ g cm}^{-2}$ as measured with the Hologic densitometer). Forty-eight percent were osteopenic at the spine, a BMD between -1 SD and -2.5 SD below the young adult value ($0.772\text{--}0.937 \text{ g cm}^{-2}$). In contrast,

none of the eumenorrheic athletes were classified as being osteoporotic and only 26% were classified as being osteopenic based on spine BMD values.

Women with elevated EDI scores had low BMD for their weight. We attempted to determine whether low BMD among women with elevated EDI scores was due to oligo/amenorrhea or whether the disordered eating had an independent effect on bone. Eight women with high EDI scores were currently eumenorrheic and had no history of amenorrhea or delayed menarche. BMD was significantly lower at the spine and was lower at the hip and whole body in this subgroup compared to eumenorrheic women with normal EDI, after adjusting for weight, body composition, age, and age at menarche. Eumenorrheic women with elevated EDI were heavier and had more body fat than all other subgroups; they also started running at a later age (18.3 ± 1.3 yr). Possibly, this group was resistant to loss of menses despite their aberrant eating because their menstrual cycles were established before they started running and/or because they were not as thin (41). It is also possible that these women have subclinical menstrual abnormalities, such as anovulatory cycles and shortened luteal phase, which have been associated with spinal bone density losses (36).

In our study population, having both disordered eating and oligo/amenorrhea was no more detrimental for bone than having either disorder alone. The numbers in some of our groups were small, however, and this observation should be verified in further studies. That there was no excess risk suggests that the two disorders share causal pathways. Both oligo/amenorrhea and disordered eating have been associated with low serum estrogen concentrations (25,49,50), which would be expected to have an adverse effect on BMD. Accordingly, disordered eating may result in estrogen deficiency or other sex hormone changes, which then may lead both to bone loss and menstrual irregularity. Menstrual irregularity and disordered eating may also contribute to bone loss, or lack of bone formation, through metabolic changes (49).

Figure 2 summarizes risk factors for low BMD and menstrual irregularity, as well as possible pathways connecting elements of the female athlete triad. Disordered eating may decrease menstruation and BMD through estrogen deficiency and through alterations of other metabolic pathways (43). Low weight is an established independent risk factor for low BMD; in this study population, women weighing less than 115 lb had fivefold-increased odds of being osteopenic at any skeletal site (OR [95% CI]: 5.3 [1.6–17.0]). Some previous studies also found an association between low weight and oligo/amenorrhea (26,6,32,45), though this study did not. Menstrual irregularity may be related to low BMD through mechanisms other than reduced estrogen (36,49). Training factors and delayed menarche have direct influences on the menstrual cycle and on BMD.

It is difficult to explain why the athletes with elevated EDI scores were heavier than the women with low EDI scores even though they reported lower energy and fat intakes. We would expect women with subclinical eating disorders to have lower weight and body fat, but this was not the case in our study. Possibly, heavier women are more prone to eating disorders

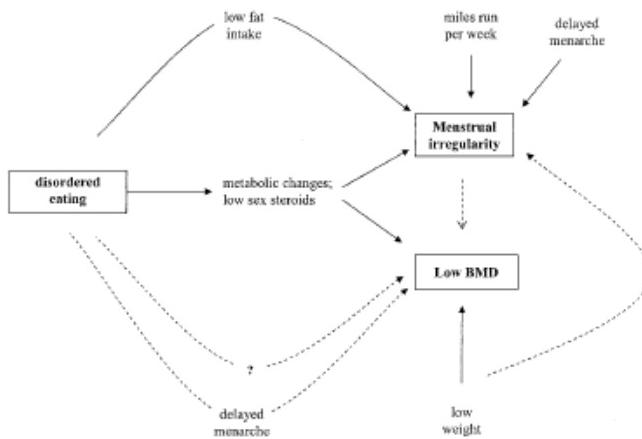


FIGURE 2—Proposed pathways among disordered eating, menstrual irregularity, and low BMD. Solid lines represent associations suggested by the current study; dashed lines represent associations suggested by previous studies.

because they are more dissatisfied with their natural body type. Some of these athletes with higher EDI scores may have had bulimic behaviors that could have explained the higher weights. Alternatively, the EDI scale may identify women in the early stages of an eating disorder but may miss women in the later stages, when they have already lost weight. We speculate that some of the women in the thinnest subgroup, the oligo/amenorrheic women with low EDI scores, may have had eating disorders but may be in denial and/or may currently be satisfied with their bodies because they have succeeded in reaching a low weight. We further recognize that the division of the population into normal EDI/elevated EDI is simplistic. There is a continuum of disordered eating behavior, but we have artificially imposed a division on that continuum. However, multiple linear regression analysis, in which we treat EDI as a continuous variable, confirms our categorical data results.

Our results are limited by the fact that menstrual status, training history, and diet were assessed by subject recall. We recognize that recall menstrual histories cannot be as accurate as those obtained by prospective record keeping. However, we believe that these menstrual histories were reasonably accurate, as the subjects were young, had short histories to recall, and, as competitive athletes, tend to be aware of their overall health. Many competitive runners keep detailed logs of their training and their miles run per week, which may have helped to minimize recall errors on the training section of our questionnaire. Finally, we recognize the limitations of food frequency questionnaires but note that the questionnaire that was employed was specifically modified to accommodate the special diets of college-aged female athletes. Prospective studies are needed confirm and further explore our findings.

Additionally, we may have missed women with subclinical menstrual abnormalities, such as anovulatory cycles and shortened luteal phase, because we assessed menstrual irregularity by questionnaire rather than laboratory testing. Measurement of serum hormone levels would have provided additional information about the

role of sex hormones. Accurate measurements of energy expenditure using doubly labeled water would have helped us to assess the role of energy balance in menstrual irregularity and low BMD. However, such measurements were outside of the scope and resources of the present study.

A further limitation of our findings is that eating attitudes and body image perception may influence the reporting of food intake (8). We cannot rule out the possibility that women with aberrant attitudes about body and food systematically underreport intake. As they are hyperconscious about their food intake, they may report what they think they should be eating rather than what they actually eat. Food frequency questionnaires, despite other limitations, may help minimize this tendency, as the total amounts of daily food, calories, and fat being reported are not readily quantifiable to the athlete.

In conclusion, we provide evidence that confirms the female athlete triad. We also conclude that the female athlete triad may be more hidden than previously realized. The women in this study were not excessively lean; indeed, amenorrheic women averaged more than 22% body fat and women with elevated EDI scores averaged more than 25% body fat. Thus, those with the triad may not be readily discernible to a coach or a physician. However, both amenorrhea and disordered eating significantly affect bone, even in the absence of the other. Because there is a high prevalence of osteopenia in this population that may have serious life-long consequences, we recommend that all competitive women endurance athletes, particularly those in sanctioned collegiate programs, receive screening for eating disorders and menstrual irregularity and education about the female athlete triad.

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The effect of oral contraceptives on bone mass and stress fractures in female
runners

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Running title: Oral contraceptives and bone health

Purpose: To determine the effect of oral contraceptives (OCs) on bone mass and stress fracture incidence in young female distance runners. **Methods:** 150 competitive female runners aged 18-26 years were randomly assigned to OCs (30 µg ethinyl estradiol and 0.3 mg norgestrel) or control (no intervention) for two years. Bone mineral density (BMD) and content (BMC) were measured yearly by dual x-ray absorptiometry. Stress fractures were confirmed by x-ray, magnetic resonance imaging, or bone scan. **Results:** Randomization to OCs was unrelated to changes in BMD or BMC in oligo/amenorrheic (n=50) or eumenorrheic runners (n=100). However, treatment-received analyses (which considered actual OC use) showed that oligo/amenorrheic runners who used OCs gained about 1% per year in spine BMD ($p<.005$) and whole body BMC ($p<.005$), an amount similar to those who regained periods spontaneously and significantly greater than those who remained oligo/amenorrheic ($p<.05$). Dietary calcium intake and weight gain independently predicted bone mass gains in oligo/amenorrheic runners. Randomization to OCs was not significantly related to stress fracture incidence, but the direction of the effect was protective in both menstrual groups (hazard ratio [95% CI]: 0.57 [0.18, 1.83]) and the effect became stronger in treatment-received analyses. The trial's statistical power was reduced by higher-than-anticipated non-compliance. **Conclusion:** OCs may reduce the risk for stress fractures in female runners, but our data are inconclusive. Oligo/amenorrheic athletes with low bone mass should be advised to increase dietary calcium and take steps to resume normal menses, including weight gain; they may benefit from OCs, but the evidence is inconclusive. **Key words:** randomized trial; amenorrhea; female athlete triad; bone density; calcium

Introduction

Paragraph 1. Female athletes with amenorrhea or oligomenorrhea have reduced bone mineral density (BMD) for their age (4,5,9,24,30). Physicians have conventionally treated amenorrheic athletes with hormone therapy or oral contraceptives (OCs) (12), but these treatments are controversial (17). Athletic amenorrhea is strongly related to disordered eating and caloric restriction (5,7, 28), and exogenous estrogens may be ineffective at improving BMD in the absence of improved nutrition and weight gain (7,9,30). Indeed, in non-athletic women with clinically apparent anorexia nervosa, randomized trials have found no effect for hormone therapy or OCs on bone (for a review of these trials, see reference 19). In amenorrheic athletes, one longitudinal study found modest skeletal benefits for hormone therapy (6), but two small randomized trials found no benefit (11,24). Longitudinal studies have also found small to modest skeletal benefits for OCs (4,22,25) and one randomized trial found that OC use reduced bone turnover in amenorrheic athletes, but no randomized trials have evaluated the impact of OCs on BMD in this population.

Paragraph 2. The effect of OCs on the BMD of eumenorrheic athletes is also unknown. Some eumenorrheic athletes have subclinical menstrual irregularities (e.g., anovulatory cycles) that are associated with an increased risk of bone loss (21), and, hypothetically, OCs might benefit this subgroup. Alternatively, eumenorrheic athletes may be similar to non-athletic premenopausal women, for whom OCs have little effect on bone (19). Lastly, OC use could be detrimental to bone health in exercising women with normal menstrual cycles. Studies from two research groups found that physically active women who used low-dose OCs (<50 µg ethinyl estradiol) had reduced BMD compared with physically

active women who did not use OCs (14,15,26) or inactive women (26). To our knowledge, there have been no randomized trials of OC use and BMD in eumenorrheic female athletes.

Paragraph 3. OC use may also protect against stress fractures in athletes, by affecting bone quality, bone turnover, or a combination of these (2), but results of previous studies are mixed. One cross-sectional and one case-control study linked OC use to a decrease in stress fracture risk (1,20), but two prospective cohort studies, in athletes (3) and female military recruits (23), found no association. There have been no randomized trials to test this hypothesis.

Paragraph 4. We conducted a randomized trial to test the effect of OC use on bone mass and stress fracture incidence in female runners. We chose to focus on running to reduce heterogeneity otherwise introduced by multiple sports, and because runners have a high frequency of both amenorrhea and stress fractures.

Materials/methods

Participants and recruitment

Paragraph 5. The study recruited 150 competitive female runners from inter-collegiate cross country teams, post-collegiate running clubs, and road races mainly in the geographic areas of Palo Alto, CA, Los Angeles, CA, Ann Arbor, MI, West Haverstraw, NY, and Boston, MA. Recruitment took place between August 1998 and September 2003. To be eligible, women had to be 18-26 years old, run at least 40 miles/week during peak training times, and compete in running races. Women were excluded if they had used OCs, other hormone therapy, or other hormonal contraception within six months before entering the study; were unwilling to be randomized to take OCs or not to take them for two years; or

had any medical contraindications to OC use. All women were required to visit a study physician or student health service staff member prior to enrollment in the study to rule out contraindications to OC use. Details of the study and testing procedures were explained to each subject, and a written, informed consent was obtained. The protocol was approved by the Institutional Review Boards of Stanford University, the University of California Los Angeles, the University of Michigan, the Helen Hayes Hospital, the Massachusetts General Hospital, the U.S. Army Medical Research and Materiel Command, and the colleges from which participants were recruited.

Randomization and intervention

Paragraph 6. Eligible women were randomly assigned to receive OCs or no intervention for an intended 2 years, stratified according to clinical site. An independent investigator who was not otherwise affiliated with the study performed the randomization using a random number table. Those assigned to take OCs received the prescription from a study physician or student health service staff member. The OC active ingredients were 30 µg ethinyl estradiol and 0.3 mg norgestrel, (Lo/Ovral, Wyeth Ayerst, 28-day pack). No placebo was used, and neither the athletes nor prescribing physician were blinded to treatment assignment, as it would be unethical to have women unsure of their contraceptive status.

Data collection and follow-up

Paragraph 7. At baseline, participants visited one of the clinical sites for bone, body composition, and physical measurements. Bone mineral density (BMD), bone mineral

content (BMC), and body composition were measured by dual energy x-ray absorptiometry (see below). Height and weight were measured using standard stadiometers and balance-beam scales, respectively (Stanford University: Harpenden stadiometer/Healthometer scale; University of California Los Angeles: Healthometer; University of Michigan: Healtometer; Helen Hayes Hospital: Measurement Concepts stadiometer/Detecto scale; Massachusetts General Hospital: Healtometer). Participants also filled out questionnaires on menstrual history, previous use of OCs, injury and stress fracture history, training regimen, diet, eating attitudes, and eating behaviors as previously described (5). Women were classified as amenorrheic, oligomenorrheic, or eumenorrheic based on the number of menses they reported having in the previous 12 months (5). Amenorrhea was defined as 0-3 cycles in the past year; oligomenorrhea was defined as 4-9 cycles in the past year; and eumenorrhea was defined as 10 or more cycles in the past year (5). Participants were asked to return to the same clinical site one year and two years later to repeat these measurements and questionnaires.

Paragraph 8. There were 124 participants (83%) who attended at least one of these follow-up appointments and 96 (64%) participants attended both, at an average of 14.4 months (median: 13.1 months) and 26.6 months (median: 25.4 months), respectively, after baseline. Three additional women provided information on stress fracture occurrence (for an average of 7.9 months after baseline), but did not return for any clinical visits. Baseline characteristics of the 23 participants with no follow-up data were similar to those with follow-up data, except that they were more likely to have a history of stress fracture prior to baseline (52% vs. 32%, p=0.05).

Paragraph 9. Between clinic visits, participants filled out a monthly calendar on which they recorded menstrual bleeding, use of OC pills, and the occurrence of stress fractures.

Ascertainment of compliance

Paragraph 10. Women in the treatment group were asked to report if and when they discontinued taking the study medication. Treatment compliance was also monitored through return of used pill packs, monthly calendars, and yearly questionnaires. If a woman reported having discontinued treatment, she was contacted by a study investigator to determine if and when OCs were discontinued and the reason why. Similarly, women in the control group were asked to contact us if they were planning to start an OC. If so, they were encouraged to take the study pill (Lo/Ovral, Wyeth-Ayerst) or a pill with a similar dose of estrogen. Compliance was also monitored on monthly calendars and yearly questionnaires. If a woman reported having started OCs, she was contacted by a study investigator to get the date of starting OCs, as well as the formulation and the reason for starting them. Among women in the control group who took OCs, the majority took Lo/Ovral or Ortho Tri-Cyclen (Ortho-McNeil Pharmaceutical, Inc., 35 µg ethinyl estradiol). The trends appeared similar with both formulations, but numbers were too small to make firm conclusions, so we combined them into a single OC group for all secondary analyses.

Ascertainment of outcomes: Bone mineral density and content

Paragraph 11. At baseline and each follow-up visit, BMD (g/cm^2) and BMC (g) at the left proximal femur, lumbar spine, and whole body, were estimated by dual energy x-ray

absorptiometry (DXA, Hologic QDR 4500A at 4 sites, QDR 2000 at one site). The coefficient of variation for measuring BMD at the hip and spine in the same person after leaving and then returning to the measuring table on the same day was 2% or less at each of the clinical sites (Stanford University: 0.9% for the lumbar spine, 0.6% for the total hip; University of California Los Angeles: 1.4% spine, 2.2% femoral neck; University of Michigan: 1.0% spine, 0.9% femoral neck; Helen Hayes Hospital: 1.2% spine, 1.4% hip; Massachusetts General Hospital: 1.0% spine, 1.4% hip). For most of the periods of data collection, machines were cross-calibrated using a circulating Hologic anthropomorphic spine phantom, and each site maintained a quality assurance program.

Ascertainment of outcomes: stress fractures

Paragraph 12. Participants were asked to record the occurrence of a possible stress fracture on a monthly calendar and also to report their occurrence to the coordinating center immediately. Participants were also queried periodically about the occurrence of stress fractures by e-mail, phone, and on their questionnaires. Fractures had to be confirmed by x-ray, bone scan, or magnetic resonance imaging to be counted in this study. All self-reported stress fractures were in fact confirmed. The study paid for the imaging as needed. We included stress fractures that occurred up to one month after the final follow-up visit.

Statistical design and analysis

Paragraph 13. We calculated that we would need 150 subjects (75 per group) to attain 80% power to detect differences in changes in BMD and stress fracture incidence between the OC group and the control group, assuming a 20% annual rate of stress fractures

in the control group (3), a 3-fold difference in stress fracture incidence (1,20), and a half-standard deviation difference in changes in BMD, and accounting for anticipated losses to follow-up and noncompliance (we anticipated that 5% of subjects would be lost to follow-up; 20% of treated subjects would discontinue OCs; and 5% of control subjects would begin OCs).

Paragraph 14. Statistical analyses were performed using the SAS statistical package, version 9.1 (SAS Institute, Cary, NC, U.S.A.). Means were compared between groups using a t-test for normally distributed variables and a Wilcoxon sum-rank test for non-normally distributed variables. Proportions were compared using a chi-square test or a Fisher's exact test, in the case of small cells. For graphing, changes in BMD, BMC, and weight were expressed as annualized percent change since baseline.

Paragraph 15. All primary outcomes were analyzed according to the intention-to-treat principle. Linear mixed-effects models were used to determine the effect of OCs on changes in BMD and BMC over time. As initially planned, all BMD and BMC analyses were stratified according to baseline menstrual status. Cox proportional hazards models were used to determine the effect of OCs on stress fracture incidence. Potential effect modifiers of the relationship between OCs and bone mass or OCs and stress fractures were evaluated by stratifying the model (for categorical variables) or by including an interaction term (for both categorical and continuous variables).

Paragraph 16. Secondary analyses were performed on the 127 women who provided follow-up data. Per-protocol analyses excluded women from the analysis at the time they switched groups. Treatment-received analyses grouped women according to their actual use of OCs, or modeled OC use as a time-dependent variable (allowing OC status to

change at the dates of starting and stopping OCs). BMD and BMC changes were analyzed by mixed models and stress fracture data were analyzed by Cox proportional hazards models. In mixed models with changes in BMD or BMC as the outcome, calcium intake was adjusted for energy intake by the residual method (27).

Results

Baseline characteristics

Paragraph 17. One-hundred fifty women were randomized to receive OCs or no intervention (Figure 1). By chance, 69 women were assigned to the OC group and 81 to the control group. The groups were well balanced on age, race/ethnicity, BMD, stress fracture history, menstrual history, weight and body composition, dietary factors, and training factors (Table 1). Amenorrhea was more common in the OC group and oligomenorrhea was more common in the control group, but these differences were not statistically significant and the groups were similar in the total proportion of athletes with menstrual irregularity (amenorrhea or oligomenorrhea).

Paragraph 18. At baseline, amenorrheic women had the lowest BMD on average (spine: 0.932 g/cm², hip: 0.937 g/cm²); oligomenorrheic women had intermediate values (spine: 0.967, hip: 0.972 g/cm²); and eumenorrheic women had the highest BMD (spine: 0.995 g/cm², hip: 0.988 g/cm²). However, these differences did not quite reach statistical significance.

Retention and adherence

Paragraph 19. Twenty-three participants (15%) withdrew or were lost to follow-up after baseline (Figure 1). Reasons for withdrawing included: geographic relocation, pregnancy, illness, and lack of time. Of the remaining 127 participants, 42 (33%) switched groups during the study—25.5% of the treatment group discontinued OCs after an average of 5.4 months of use and 38.9% of the control group started taking them at an average of 11.3 months into the study (Table 2). Four women in the control group and one woman in the treatment group switched groups twice. The reasons women gave for stopping OCs included (in decreasing order of frequency): fear of weight gain or perceived weight gain, side effects (irritability, abdominal symptoms, nausea, fatigue, or unspecified), and fear of detriment to athletic performance. The reasons control women gave for starting OCs included (in decreasing order of frequency) to: regulate periods, alleviate menstrual symptoms and cramps, prevent pregnancy, treat acne, and treat allergies.

Paragraph 20. Women who stopped taking OCs had significantly lower percent body fat, fewer menstrual periods per year, and more disordered eating than women who adhered to OCs (Table 2). Amenorrheic women were the least likely to comply with taking OCs: of eight amenorrheic women who were assigned to OCs, only one took them through the entire study (of the remaining seven, two were lost to follow-up, five discontinued OCs within two months, and one discontinued OCs after 1.5 years). In the control group, women who self-initiated OC use were less likely than control adherent women to have a history of stress fractures prior to baseline.

Primary analysis: Bone mineral content and density

Paragraph 21. The effect of OCs on bone mass was similar across the clinical sites, so we combined the data from the sites, retaining site as a covariate in all models. Results for spine and hip BMD were similar to results for spine and hip BMC; for comparability with previous studies, we report the BMD results for these sites.

Paragraph 22. We found that randomization to OCs had no effect on changes in bone mineral content or density—with one exception: in the oligomenorrheic group, total hip BMD was significantly reduced ($p=0.04$) in the OC group compared with the control group (Table 3). This finding may be the result of chance due to multiple comparisons and small numbers. Following correction for multiple comparisons with a Hochberg correction (16), this difference was no longer statistically significant at the .05 level.

Paragraph 23. Regardless of treatment assignment, bone changes were strongly related to initial menstrual status. Overall, the amenorrheic and oligomenorrheic groups had significant increases in spine BMD and whole body BMC, with the largest gains occurring in the amenorrheic group. Eumenorrheic women had a small but significant increase in whole body BMC (6.4 ± 2.6 g/year, $p<.05$), but no changes in hip or spine BMD.

Paragraph 24. We found no interactions between randomization status and age, BMD, weight, weight changes, body composition, disordered eating, calcium intake, or miles run per week with respect to bone outcomes.

Secondary analyses: Bone mineral content and density

Paragraph 25. One hundred and twenty-four women had at least one follow-up DXA and were included in secondary analyses. We combined the amenorrheic and oligomenorrheic groups for these analyses because the groups gave similar results when

analyzed separately, but the amenorrheic group was too small (n=10) to yield precise estimates in multivariate analyses.

Paragraph 26. Per-protocol and treatment-received analyses gave similar results to the intention-to-treat analysis (data not shown), except we did not find a negative effect of OCs on hip BMD in oligo/amenorrheic women. For treatment-received analyses, we classified women as being in the OC group if they had used OCs for at least six months during the study. We used a cutoff of six months because it may take this long for OCs to affect bone mineral density. We repeated all analyses using an alternate cutoff of three months or modeling OC use as a time-dependent variable, and found similar results (data not shown).

Paragraph 27. Fourteen of the oligo/amenorrheic women (4 amenorrheic and 10 oligomenorrheic) regained their periods spontaneously (had 10 or more periods in the year prior to their final measurement), without the help of OCs. When we divided oligo/amenorrheic women into those who had used OCs (for at least six months), those who regained their periods spontaneously, and those whose cycles never normalized, we found that OC users gained significantly more whole body BMC and spine BMD than women who remained oligo/amenorrheic (Figure 2, Table 4). The gain in bone mass among OC users did not differ statistically from women who regained periods spontaneously. On average, both groups gained about 1% per year in whole body BMC and spine BMD, whereas women who remained oligo/amenorrheic neither gained nor lost bone. Average weight gain was (non-significantly) higher in the OC group than the other two groups during the first year of the study (Figure 2), but adjustment for weight changes did not remove the effect of OCs (Table 4). Adjustment for changes in body composition gave similar results (data not shown).

Paragraph 28. In oligo/amenorrheic women, weight gain independently predicted gains in spine BMD and whole body BMC, and showed a trend at the hip ($p<.10$). Gains in fat mass also independently predicted gains in spine and hip BMD and whole body BMC, but gains in lean mass predicted gains only in whole body BMC (data not shown). Since changes in fat mass and weight were highly correlated ($r=.84$, $p<.0001$), we chose to include weight in the final model because it is a more clinically accessible measure. Higher dietary calcium intake also predicted gains in whole body BMC and hip BMD in oligo/amenorrheic women.

Paragraph 29. In eumenorrheic women, weight gain was not associated with bone changes, but dietary calcium intake was associated with increases in hip BMD ($p<.05$), and showed a trend for whole body BMC ($p<.10$) (Table 4).

Primary analysis: stress fractures

Paragraph 30. Eighteen runners had at least one stress fracture during the study in the tibia, foot, femur, or pelvis (Table 5). Six occurred in the group randomized to OCs (5.8 per woman-year) and 12 in the group randomized to control (9.2 per woman-year) (Table 6). After adjusting for baseline menstrual status, clinical site, age, prior stress fracture, and spine BMD (the latter two variables were strongly related to fracture risk) in a Cox proportional hazards model, we found that randomization to OCs yielded a non-significant 43% decrease in the rate of stress fracture. This effect was similar across the different clinical sites and across baseline menstrual groups; the hazard ratio (and 95% confidence interval) for eumenorrheic women was: 0.56 (0.14, 2.22), and for oligo/amenorrheic women was: 0.60 (0.06, 5.83).

Paragraph 31. Women who were oligo/amenorrheic at baseline were not at increased risk of fracture compared with women who were eumenorrheic at baseline (HR: 1.20); however, the majority of oligo/amenorrheic women regained menstrual regularity during the trial. A small group of women who remained oligo/amenorrheic (n=11) or became so during the study (n=2) had a non-significant increase in fracture risk (HR [95% CI]: 2.71 [0.70-10.60]).

Paragraph 32. We did not find interactions between randomization status and age, low BMD, weight, weight changes, body composition, past menstrual irregularity, disordered eating, calcium intake, or miles run per week with respect to stress fractures, though we had limited statistical power to detect interactions.

Paragraph 33. Four women had a second stress fracture during the study (three in the control group and one in the treatment group), but this was too few to evaluate statistically.

Secondary analyses: stress fractures

Paragraph 34. When we excluded non-adherent women from our analysis on the date at which they switched groups, OCs appeared more protective, but did not reach statistical significance (Table 6). We then modeled OC use as a time-dependent variable to ensure that we were only counting OC treatment that occurred prior to each fracture. When women were taking OCs (and had been on them at least a month), OC use appeared to be significantly protective (HR [and 95% CI]: 0.23 [0.06,0.86]). However, four fractures occurred in the control group within the first three months of the study, and it is unclear if these fractures can be attributed to anything other than chance. Excluding these fractures by

requiring OC use of at least 3 months reduced the magnitude of the effect slightly and also reduced our statistical power (HR [and 95% CI]: 0.40 [0.11, 1.50]).

Adverse events

Paragraph 35. There were no serious adverse events in the trial. Five women discontinued OCs citing irritability, abdominal symptoms, nausea, fatigue, or unspecified side effects.

Discussion

Paragraph 36. We found that randomization to OCs had no effect on BMD or BMC in oligo/amenorrheic or eumenorrheic female runners, and yielded a 43 percent reduction (not statistically significant) in rate of stress fractures across menstrual groups. The trial's statistical power was diminished by non-compliance: 38.9 percent of women in the control group started taking OCs and 25.5 percent of women in the treatment group stopped taking them (among those with follow-up data). Additionally, power was reduced because 38 percent of oligo/amenorrheic runners in the control group resumed normal menses spontaneously. We confirm the difficulties of doing a definitive trial of OCs in female athletes (11).

Paragraph 37. Contrary to previous reports (14,15,26), we did not find that use of low-dose OCs was detrimental to bone mineral density levels in eumenorrheic female athletes. Some of these previous reports were cross-sectional studies (14,15), which cannot establish the direction of causality and may be confounded by reasons for use of OCs. Because of our choice of study population, we cannot rule out a negative effect of OC use

for inactive women who begin an exercise program (26) or for athletes younger than 18 (14).

Paragraph 38. In our treatment-received analyses, we found that oligo/amenorrheic runners who took OCs for at least six months gained more spine BMD and whole body BMC than runners who remained oligo/amenorrheic, and this association was independent of changes in weight or body composition. The magnitude of the effect—approximately 1% annual gains—was similar to that of regaining periods spontaneously or gaining 5 kg. However, we cannot conclude that OCs per se caused these gains. Women who dropped out of the OC group were more likely to be amenorrheic and have disordered eating, two factors that predispose to continued bone loss or lack of bone growth. Oligo/amenorrheic runners who adhered to or started on OCs may have been concerned about their bone health and thus actively trying to improve it in other ways not discernible in this study.

Paragraph 39. Results of previous studies of estrogen supplementation and BMD in amenorrheic athletes have been mixed and may be complicated by the use of different formulations and doses of hormones. Longitudinal cohort studies of OCs (30-35 µg ethinyl estradiol [4,22,25] or hormone therapy (0.625 mg conjugated estrogen or 50 µg estradiol patch [6]) have found small to modest positive effects on BMD in amenorrheic athletes, but these studies may be confounded by other factors associated with the choice to take hormones. Two randomized trials failed to find an effect of hormone therapy (Premarin/Provera and 2 mg estradiol/1 mg estriol, respectively) in 24 amenorrheic ballet dancers (24) and 34 oligo/amenorrheic runners (11). However, similar to our findings with OCs, the latter trial did find a significant benefit for using hormones compared with remaining oligo/amenorrheic in treatment-received analyses.

Paragraph 40. Our results confirm previous findings that spontaneous recovery of menses benefits the skeleton (8,11,18). In our study, it was unclear why some runners spontaneously resumed normal menses and others did not, and the reasons are likely heterogeneous. Previous researchers have found that decreased training, increased caloric intake, and weight gain predict spontaneous resumption of menses (8,18). We found that, on average, women who spontaneously regained menses had a trend toward higher caloric intake than women who remained oligo/amenorrheic, but this translated to only slightly higher average gains in weight and fat mass. We speculate that small improvements in energy balance and eating patterns may normalize menstrual periods without substantial weight gain.

Paragraph 41. We confirm previous findings that weight gain is an important independent predictor of bone mass gain in oligo/amenorrheic athletes (18); weight gain was associated with increases in whole body BMC, spine BMD, and hip BMD. Fat mass gains were more predictive of changes in BMD and BMC than lean mass gains.

Paragraph 42. Dietary calcium intake (controlled for energy intake) predicted gains in whole body BMC and hip BMD in both oligo/amenorrheic and eumenorrheic athletes, with a stronger effect in oligo/amenorrheic women. We found no effect for calcium supplementation, but this variable was imprecisely measured, and use of supplements was sporadic in this population. One previous cross-sectional study found a relationship between dietary calcium intake and BMD (29), but these estimates were not adjusted for energy intake. We believe the present study is the first longitudinal study to show that dietary calcium intake is important for continued skeletal mineralization in young adult female runners.

Paragraph 43. Whole body BMC was significantly increasing over the course of the study in all menstrual groups, thereby indicating continued skeletal mineralization in this age group. Amenorrheic and oligomenorrheic women who recovered their periods (through OCs or spontaneously) gained whole body BMC and spine BMD (but not hip BMD) at a faster rate than eumenorrheic women. This is promising in that it suggests a catch-up effect whereby previously amenorrheic and oligomenorrheic athletes with reduced BMD can gain bone in the third decade of life (9).

Paragraph 44. This is the first randomized trial to test whether OCs can protect young female athletes against stress fractures. Our results are inconclusive, but show a trend toward protection. In our intention-to-treat analysis, there was a non-significant 43% reduction in stress fracture incidence among women randomized to OCs. The magnitude of the effect was similar in eumenorrheic and oligo/amennorheic runners. Follow-up, but not baseline, menstrual irregularity was associated with a non-significant increase in fracture risk.

Paragraph 45. The effect of OCs on stress fractures became stronger in both per-protocol and treatment-received analyses. In our treatment-received analysis, women were significantly protected against fractures (by 77%) whenever they were taking OCs, though this estimate was weakened when we excluded fractures that occurred early in the trial (58% reduction in risk, $p=.20$). Our finding may be due to chance or bias. We found that women who switched from the control group to OC use were less likely to have a history of stress fractures prior to joining the study. Thus, the type of woman runner who is willing to continue on or chooses to take OCs may be less prone to fracture for other reasons.

Paragraph 46. OCs may protect against stress fractures by suppressing bone turnover (25). During bone remodeling, bone resorption precedes bone formation, temporarily leaving skeletal sites weakened and more vulnerable to fracture (2). OCs may also protect against fracture through cumulative effects on BMD (2), but we found no evidence of this in our trial. Finally, OCs may be acting on some other aspect of bone quality that affects fracture risk.

Paragraph 47. Our findings are consistent with two previous observational studies that found protective effects of similar magnitude. In a case-control study by Myburgh et al. (20), current use of OCs was associated with a 76% reduction in the odds of stress fracture; in a cross-sectional study by Barrow and Saha (1), ever use of OCs (for at least one year) was associated with a 59% reduction in risk of ever having had a fracture. Our findings differ from two prospective cohort studies that reported no benefit for OCs in track and field athletes and female military recruits (3,23).

Paragraph 48. Even if OCs confer benefit, women at the highest risk of severe bone deficits and stress fractures may be unwilling to take them. The amenorrheic women in our study had the lowest BMD and were the least willing to take OCs; only one of eight amenorrheic women assigned to OCs took them for the entire study period. Women with disordered eating, considered the precipitating factor in the female athlete triad, were also less likely to continue taking OCs, possibly driven by fear of weight gain.

Paragraph 49. Our study highlights the difficulty of conducting a randomized trial of OC use in this population. Recruitment for this study took more than five years. Women have strong personal preferences regarding OC use and are reluctant to leave this decision to chance.

Paragraph 50. Even though this is the largest randomized trial yet of OCs in female athletes (and the largest in oligo/amenorrheic athletes), the trial was likely underpowered for both BMD and stress fracture outcomes, similar to the findings of Gibson et al. (11). Our original sample size calculations greatly underestimated the number of women in the control group who would switch to OCs during the trial, and we did not account for the women in the oligo/amenorrheic group who would spontaneously regain periods and thus obscure our ability to see effects. Despite our best efforts, 15 percent of the study sample provided no follow-up data, which was slightly higher than initially anticipated. The rate of stress fracture in the control group was also lower than anticipated. Based on our results, we estimate that we in fact had only 20 percent power to detect an effect of OCs on stress fractures in our intention-to-treat analysis. We estimate that 900 runners would be required to have 80 percent power to detect an effect of OCs on stress fractures in a two-year trial of female runners of any menstrual status. From our study, it is unclear if an adequately powered trial for the effect of OCs on BMD (in oligo/amenorrheic athletes) is even possible; effects may be completely obscured regardless of sample size because of the high rates of women switching groups or spontaneously regaining menses. Based on their data, Gibson et al. previously estimated that 1150 oligo/amenorrheic athletes would be needed (11); given the difficulties that we had recruiting for a trial of 150 runners of any menstrual status, we believe it would be extremely difficult to enroll this many oligo/amenorrheic athletes.

Paragraph 51. We used an oral contraceptive with 30 µg ethinyl estradiol and 0.3 mg norgestrel. We cannot rule out that different dosages, different routes of administration of hormones, or a different ratio of estrogen to progestin might have more beneficial effects

on the skeleton. For example, isolated case reports in amenorrheic women suggest transdermal estrogen may confer more benefits to bone than oral estrogen (13,30).

Paragraph 52. We did not use a placebo control because of ethical considerations and the high probability of unblinding, as most women would have figured out whether or not they were on OCs by the timing of their menstrual cycles. We did not measure serum hormone concentrations or markers of bone turnover, which may have added information to the study, because these measurements were outside of the study's scope and resources. Because of our lack of hormone data, we cannot rule out that some cases of menstrual irregularity were due to mechanisms other than hypothalamic suppression; such cases could have contributed to our failure to find an effect for OCs. We did not have data on the severity of stress fracture injury, which may have limited our ability to see effects. We included stress fractures from multiple skeletal sites in our analysis; since OCs may differentially affect different skeletal sites, this may also have obscured our ability to see effects. Finally, our results may not apply to athletes in other sports, since only runners were considered.

Paragraph 53. In summary, we found that OC use is not detrimental to BMD or BMC in female runners and may protect against stress fractures. Our data suggest that oligo/amenorrheic athletes with low BMD should be advised to gain weight, increase dietary calcium intake, and consider taking OCs if they are unable to establish regular menses on their own. However, we underscore that no clinical trials (including our own) have definitively shown that hormone therapy or OCs increase (or prevent loss of) BMD or BMC in this group. We conclude that it will be difficult to conduct a randomized trial that definitively answers this question.

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TABLE 1. Mean \pm one standard deviation or percentage (number) with selected characteristic at baseline, by treatment randomization.

	Treatment randomization	
	Oral contraceptives (n=69)	Control (n=81)
Age (yr)	22.3 \pm 2.7	21.9 \pm 2.6
Race/ethnicity		
White	82.6% (57)	82.7% (67)
Asian/Pacific Islander	4.4% (3)	9.9% (8)
Hispanic	7.3% (5)	3.7% (3)
Black	2.9% (2)	0% (0)
Other	2.9% (2)	3.7% (3)
Clinical site		
Stanford	53.6% (37)	43.2% (37)
Boston	17.4% (12)	21.0% (17)
Los Angeles	15.9% (11)	21.0% (17)
New York	10.1% (7)	8.6% (7)
Michigan	2.9% (2)	6.2% (5)
Hip BMD (g/cm ²)	0.986 \pm 0.119	0.975 \pm 0.114
Spine BMD (g/cm ²)	0.979 \pm 0.098	0.985 \pm 0.112
Whole body bone mineral content (g)	2171 \pm 312	2146 \pm 279
History of one or more stress fractures	36.2% (25)	33.3% (27)

Age at menarche (yr)	13.1 ± 1.4	13.0 ± 1.5
Total lifetime menstrual periods (no. cycles)	69 ± 28	67 ± 30
Menses in past year (no. cycles)	9.4 ± 3.8	9.5 ± 3.1
Irregular menses		
Amenorrhea *	11.6% (8)	6.2% (5)
Oligomenorrhea †	18.8% (13)	29.6% (24)
Ever used oral contraceptives	43.5% (30)	40.7% (33)
Height (cm)	165.9 ± 6.6	165.4 ± 6.1
Weight (kg)	58.2 ± 7.3	58.1 ± 6.6
Body mass index (kg/m^2)	21.1 ± 1.9	21.3 ± 2.0
Body fat (%)	22.7 ± 5.2	23.3 ± 5.4
Daily caloric intake ($\text{kcal}\cdot\text{day}^{-1}$)	2250 ± 893	2302 ± 988
Dietary calcium intake ($\text{mg}\cdot\text{day}^{-1}$)	1394 ± 829	1412 ± 670
Total eating disorder inventory score‡	14.7 ± 14.7	10.6 ± 11.8
Age started running competitively (yr)	14.1 ± 3.8	14.3 ± 3.3
Average distance run per week, past year (miles)	34.8 ± 10.5	34.8 ± 11.4

* 0-3 menstrual periods in the year before baseline.

† 4-9 menstrual periods in the year before baseline.

‡ Total eating disorder inventory score, which can range from 0-69, is the sum of the scores from the anorexia, bulimia, and body dissatisfaction subscales, Garner and Olmstead (10).

TABLE 2. Selected follow-up measures and baseline characteristics according to intervention adherence (where follow-up data were available).

Characteristic	Adherence			
	Adherent to treatment (n=41)	Switched from treatment to control (n=14)	Adherent to control (n=44)	Switched from control to treatment (n=28)
<u>Follow-up measure</u>				
Time in study (months)	24.2 ± 4.7	24.7 ± 8.5	24.0 ± 6.4	25.6 ± 8.3
Time switched groups (months into study)	--	5.4 ± 5.6	--	11.3 ± 10.3
Oral contraceptive use (months)	24.2 ± 4.7	5.4 ± 5.6	0	14.4 ± 9.1
<u>Baseline characteristic</u>				
Age (yr)	22.0 ± 2.7	22.2 ± 2.8	22.1 ± 2.6	21.9 ± 2.8
Hip BMD (g/cm ²)	0.994 ± 0.132	0.962 ± 0.088	0.991 ± 0.115	0.977 ± 0.108
Spine BMD (g/cm ²)	0.984 ± 0.104	0.960 ± 0.103	1.00 ± 0.115	0.985 ± 0.109
Whole body bone mineral content (g)	2157 ± 340	2192 ± 226	2166 ± 243	2181 ± 330

History of one or more stress fractures	34.2% (14)	28.6% (4)	38.6%(17)	17.9% (5)*
Menses in past year (no. cycles)	10.8 ± 2.3	6.3 ± 5.1†	9.6 ± 2.9	9.4 ± 3.4
Irregular menses‡				
Amenorrhea	2.4% (1)	35.7% (5)§	4.6% (2)	7.1% (2)
Oligomenorrhea	14.6% (6)	21.4% (3)	36.4% (16)	25.0% (7)
Weight (kg)	58.6 ± 7.6	57.2 ± 5.4	58.5 ± 6.4	58.4 ± 6.6
Body fat (%)	23.7 ± 4.8	19.5 ± 6.1	23.6 ± 5.5	23.0 ± 5.1
Total eating disorder inventory score**	10.9 ± 11.2	17.4 ± 16.2	12.0 ± 12.4	10.0 ± 11.9
Evidence of prior or current disordered eating^	26.8% (11)	57.1% (8)#+	31.8% (14)	32.1% (9)

*p=.06, differs from control adherent group, chi-square test

†p<.005, differs from treatment adherent group, Wilcoxon sum-rank test

‡Amenorrhea was defined as 0-3 periods in the year before baseline; oligomenorrhea was defined as 4-9 periods in the year before baseline

§ p<.005, differs from treatment adherent group, Fisher's exact test

| p<.05, differs from treatment adherent group, ttest

**Total eating disorder inventory (EDI) score, which can range from 0-69, is the sum of the scores from the body dissatisfaction, anorexia, and bulimia subscales, Garner and Olmstead (10).

TABLE 3. Annual rate of change* in spine and hip BMD (BMD) and whole body bone mineral content (BMC) by treatment randomization, stratified on initial menstrual status.

	Whole body BMC (g/year ± SE)	Spine BMD (g/cm ² /year ± SE)	Hip BMD (g/cm ² /year ± SE)
<u>Amenorrheic[†]</u>			
Treatment (n=8)	16.1 ± 10.3	.0197 ± .0036 [§]	.0050 ± .0040
Control (n=5)	28.9 ± 9.9	.0138 ± .0049	.0052 ± .0054
Treatment vs. Control	-12.8 ± 12.4	.0060 ± .0061	-.0002 ± .0067
<u>Oligomenorrheic[‡]</u>			
Treatment (n=13)	23.2 ± 10.4	.0019 ± .0037	-.0096 ± .0033 ^{**}
Control (n=24)	15.3 ± 7.4	.0076 ± .0026 ^{**}	.0012 ± .0023
Treatment vs. Control	8.1 ± 12.8	-.0056 ± .0045	-.01076 ± .0041
Eumenorrheic			
Treatment (n=48)	9.9 ± 3.9	.0022 ± .0019	.0013 ± .0017
Control (n=52)	3.7 ± 3.4	.0002 ± .0016	-.0023 ± .0015
Treatment vs. Control	6.2 ± 5.2	.0020 ± .0025	.0035 ± .0022

* From linear mixed models, adjusted for age and clinical site.

[†] Amenorrhea was defined as 0-3 menses over the past 12 months.

[‡] Oligomenorrhea was defined as 4-9 menses over the past 12 months.

[§] p<.0001, rate of change differs from 0.

[|]p<.05, rate of change differs from 0.

^{**}p<.01, rate of change differs from 0.

TABLE 4. Treatment-received analyses: Adjusted annual rates of change in whole body bone mineral content (BMC) and spine and hip bone mineral density (BMD) among women with at least one follow-up DXA measurement, stratified on initial menstrual status.*

	Whole body BMC (g/year ± SE)	Spine BMD (g/cm ² /year ± SE)	Total hip BMD (g/cm ² /year ± SE)
<u>Oligo/amenorrheic[†] (n=41)</u>			
Used oral contraceptives for at least 6 months (n=16) vs.	26.8 ± 11.3 [‡]	.0103 ± .0043 [‡]	.0068 ± .0043
remained oligo/amenorrheic (n=11)			
Regained periods spontaneously (n=14) vs.	34.9 ± 11.5 [‡]	.0113 ± .0043 [‡]	.0035 ± .0043
remained oligo/amenorrheic (n=11)			
Baseline calcium intake (per 1 standard deviation increase) [§]	10.6 ± 4.9 [‡]	.0020 ± .0018	.0048 ± .0017 [‡]
Weight change (per 5 kg increase)	21.3 ± 8.8 [‡]	.0126 ± .0033	.0063 ± .0033 ^{**}
<u>Eumenorrheic (n=83)</u>			

Used oral contraceptives for at least 6 months (n=50) vs. did not use oral contraceptives for at least 6 months (n=33)	5.9 ± 5.6	.0027 ± .0027	.0034 ± .0024
Baseline calcium intake (per 1 standard deviation increase) [§]	$4.9 \pm 2.7^{**}$.0020 ± .0027 ± .0011 [‡]	0.0013
Weight change (per 5 kg increase)	-3.6 ± 10.3	.0060 ± .0049	.0060 ± .0043
*Annual rates of change are estimated from linear mixed models, adjusted for clinical site, age, baseline weight, and all other predictors shown in the table.			
†Oligo/amenorrhea was defined as 0-9 menses in the year before baseline.			
[‡] p<.05, rate of change differs from 0.			

TABLE 5 Distribution of stress fractures* by site and mode of diagnosis.

Site of fracture	Diagnostic test	Number
Tibia	Bone scan	9
Tibia	X-ray	1
Foot	X-ray	3
Foot	Bone scan	1
Foot	MRI	1
Femur	MRI	2
Pelvis	X-ray	1

*Four women had two stress fractures during this study; only their first stress fractures are included in this table.

Table 6. Effect of oral contraceptives on stress fracture according to type of analysis

Analysis	Oral contraceptives (n=69)	Control (n=81)	Hazard Ratio (95%CI)*
<u>Intention-to-treat analysis</u>			
Number of fractures	6	12	
Time to fracture or censoring in months (mean \pm SD)	18.1 \pm 11.4	19.4 \pm 11.2	
Rate of fracture, per woman-year	5.8	9.2	0.57 (0.18,1.83)
<u>Per-protocol analysis[†]</u>			
Number of fractures	5	11	
Time to fracture or censoring in months (mean \pm SD)	14.6 \pm 11.5	14.7 \pm 11.3	
Rate of fracture, per woman-year	6.0	11.1	0.40 (0.11,1.49)
<u>Treatment-received analyses</u>			
Took oral contraceptives \geq 1 month and still taking them [‡]		0.23 (0.06,0.86)**	
Took oral contraceptives \geq 3 months and still taking them [§]		0.42 (0.11,1.57)	

*Adjusted by Cox proportional hazards model for age, clinical site, baseline menstrual status, baseline spine BMD, and prior fracture. The Hazard ratios are for the oral contraceptive group compared with the control group.

†This analysis censors women who switched groups at the time of switching.

‡ Time-dependent variable that considers a woman to be in the oral contraceptive group only after she has taken them for at least 1 month and has not stopped taking them.

§Time-dependent variable that considers a woman to be in the oral contraceptive group only after she has taken them for at least 3 months and has not stopped taking them.

Figure Legends

FIGURE 1. Flow of participants through the trial.

FIGURE 2. Annualized mean percent change in whole body bone mineral content (BMC), spine and hip bone mineral density (BMD), and weight among oligo/amenorrheic women according to follow-up menstrual status (graph displays mean \pm one standard error of the mean):

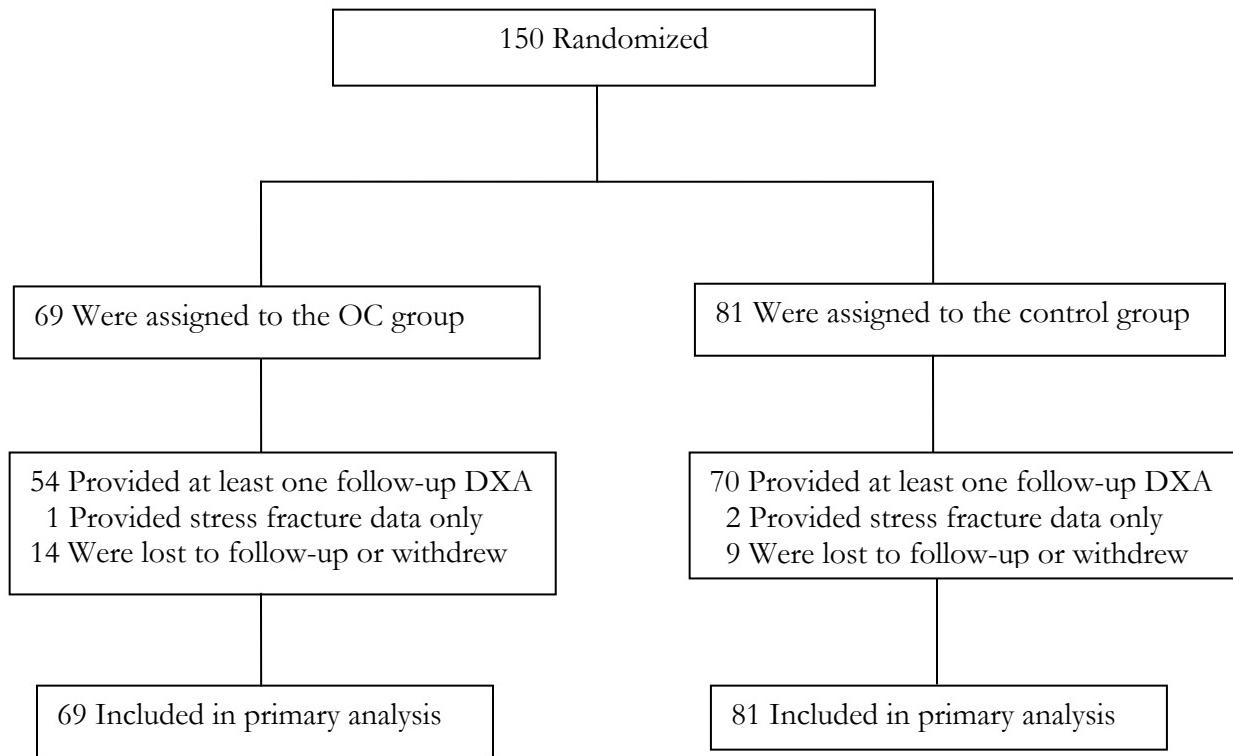
●=Used oral contraceptives at least 6 months (n=16)

▲=Spontaneously regained menses (n=14)

x=Remained oligo/amenorrheic (n=11)

* $p < .05$, different than women who remained oligo/amenorrheic, mixed models

FIGURE 1



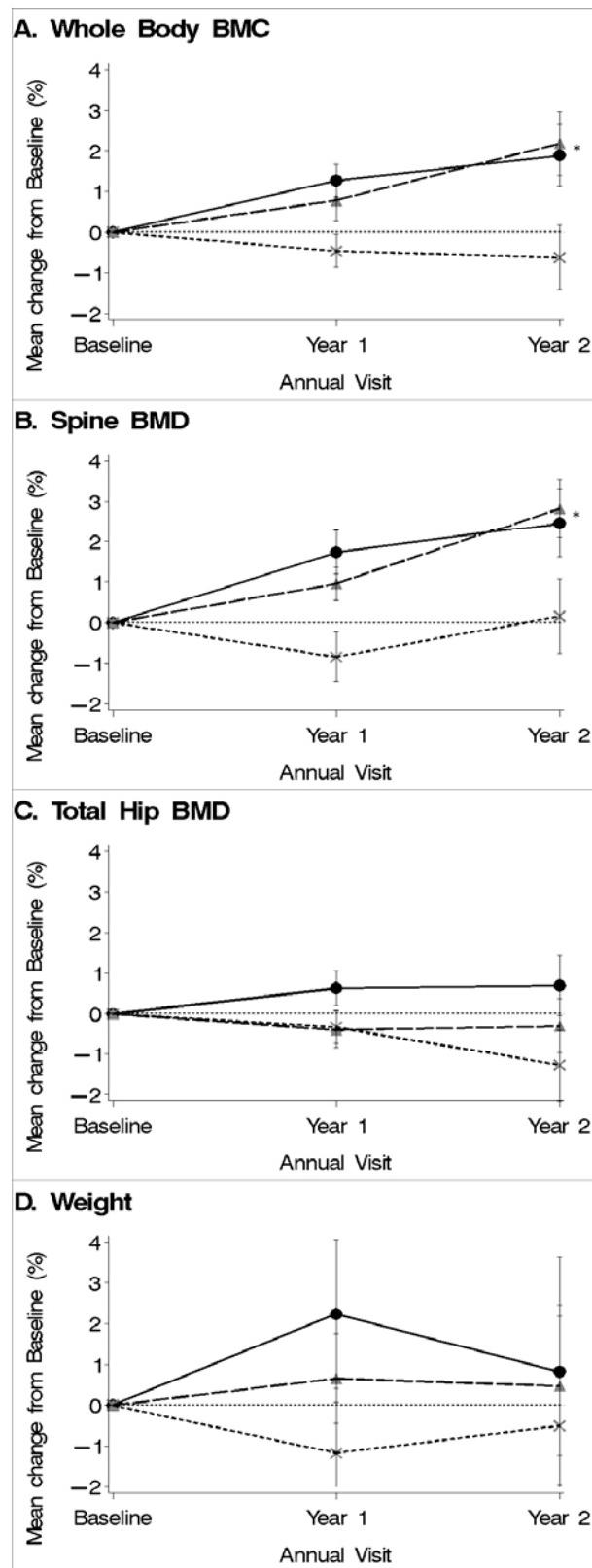


FIGURE 2

Risk Factors for Stress Fracture among Young Female Cross-Country Runners

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Running title: Stress Fractures in Young Female Runners

ABSTRACT

Purpose: To identify risk factors for stress fracture among young female distance runners. **Methods:** Participants were 127 competitive female distance runners, aged 18-26, who provided at least some follow-up data in a randomized trial among 150 runners of the effects of oral contraceptives on bone health. After completing a baseline questionnaire and undergoing bone densitometry, they were followed an average of 1.85 years. **Results:** Eighteen participants had at least one stress fracture during follow-up. Baseline characteristics associated ($p < 0.10$) in multivariate analysis with stress fracture occurrence were one or more previous stress fractures (rate ratio [RR] [95% confidence interval] = 6.42 (1.80-22.87), lower whole-body bone mineral content (RR = 2.70 [1.26-5.88] per one standard deviation [293.2 grams] decrease), younger chronologic age (RR = 1.42 [1.05-1.92] per one year decrease), lower dietary calcium intake (RR = 1.11 [0.98-1.25] per 100 mg decrease), and younger age at menarche (RR = 1.92 [1.15-3.23] per one year decrease). Although not statistically significant, a history of irregular menstrual periods was also associated with increased risk (RR = 3.41 [0.69-16.91]). Training-related factors did not affect risk. **Conclusion:** The results of this and other studies indicate that risk factors for stress fracture among young female runners include previous stress fractures, lower bone mass, and, although not statistically significant in this study, menstrual irregularity. More study is needed of the associations between stress fracture and age, calcium intake, and age at menarche. Given the importance of stress fractures to runners, identifying preventive measures is of high priority. **Key words:** BONE MASS; EPIDEMIOLOGY; FEMALE ATHLETES; LONG DISTANCE RUNNERS

INTRODUCTION

Paragraph 1. Stress fractures are common among young female competitive athletes, especially in track and field (17). Reported one-year incidence rates in competitive track and field athletes have ranged from 8.7% (22) to 21.1% (5) in females and males combined, the variation probably depending in part on the sensitivity of the methods used to detect stress fractures. Incidence rates appear to be similar in female and male track and field athletes (5, 22). It is generally agreed that current or past menstrual irregularity is a risk factor in female athletes (2, 6, 7, 9, 21, 22). Results from studies of female or female and male athletes are contradictory regarding the associations of stress fractures with age (4), lower bone mineral density or lean body mass (6, 7, 9, 22), late age at menarche (4, 6, 7, 9, 21), not using oral contraceptives (4, 7, 21), low body weight (7, 22), disordered eating (6, 7, 22), and low calcium and dairy product intake (6, 7, 21). Individual studies have reported leg length discrepancy (6), low dietary fat intake (6), and a history of stress fracture (22) to be risk factors, but confirmation in other investigations is needed. Many of these results are based on small numbers of study subjects, some have collected information retrospectively, and most do not use multivariate methods of statistical analysis to determine which of these attributes are independent predictors of stress fracture. A recent review in fact concluded that data regarding the epidemiology of stress fractures in athletes are “lacking,” except that stress fractures usually occur among those participating in sports with repetitive weight-bearing activity (29). Also, risk factors may not be the same for all athletes, so studies focusing on specific sports may provide particularly useful information for participants in that sport.

Paragraph 2. Studies of stress fracture in female or female and male military recruits and trainees have also produced somewhat inconsistent and tentative results. Possible risk factors include increasing age (19, 27), a small thigh girth (1), lower aerobic fitness (24), no or only a small amount of lower extremity weight training in the past year (24), lack of menstrual cycles in past year (24), and, in a large prospective study (19), lower bone mineral density, weight loss, alcohol consumption of more than 10 drinks per week, cigarette smoking, weight bearing exercise, lower adult weight, corticosteroid use, use of depo-medroxyprogesterone acetate, and lack of past regular exercise. Two retrospective studies have reported no association between stress fracture occurrence and bone mineral density or bone mineral content (1, 10), and a few studies have found no association with menstrual frequency or age at menarche (1, 19), calcium intake or dairy food consumption (10, 19), and eating disorders (1). In addition to methodologic limitations in some of these studies, risk factors in military recruits and trainees may have limited relevance to women who have been running competitively for several years.

Paragraph 3. In this paper we use data collected during the course of a randomized trial of the effect of oral contraceptives on bone health to identify other factors that predict stress fractures in young female long-distance runners. Results of the randomized trial are presented in a companion paper.

METHODS

Paragraph 4. Study Population: The study population for these analyses consists of 127 competitive female cross-country runners between the ages of 18-26 years at baseline who participated in a randomized trial to examine whether use of oral contraceptives protects against loss of bone mass and stress fracture occurrence. Recruitment took place between August 1998 and September 2003. One hundred fifty runners had been recruited for the trial from intercollegiate cross-country teams, post-collegiate running clubs, and road race participants, of whom 127 (85%) provided some follow-up information. Of these, 57 were collegiate runners and 70 post-collegiate runners. At the time of recruitment, most lived in the vicinities of the sites at which bone densitometry was undertaken: Stanford CA, Los Angeles CA, West Haverstraw NY, Ann Arbor MI, and Boston MA. To be eligible, women had to run at least 40 miles per week during peak training times, had to compete in races, could not have used oral contraceptives or other hormonal contraceptives within six months of entering the study, and had to be willing to be randomized and to have no contraindications to oral contraceptive use. The size of the study population was based on the number needed to provide adequate statistical power for the randomized trial, not for the comparisons presented in this paper. Details of the study and testing procedures were explained to each subject, and a written, informed consent was obtained. The protocol was approved by the Institutional Review Boards of Stanford University, the University of California Los Angeles, the University of Michigan, the Helen Hayes Hospital, the Massachusetts General Hospital, the U.S. Army Medical Research and Materiel Command, and the colleges at which participants were recruited.

Paragraph 5. Data Collected at Baseline: At each of the five clinical sites, height and weight were measured using standard stadiometers and balance-beam scales, respectively. Body mass index (BMI) (kilograms per meter²) was calculated from these measurements. Body composition (lean body mass and fat mass) and bone mineral content (grams) and bone mineral density (grams/centimeter²) at the left proximal femur, spine, and whole body and were measured by dual energy x-ray absorptiometry (DXA, Hologic QDR 4500A at 4 sites, QDR 2000W at one site). The coefficient of variation for measuring the bone mineral density at the hip and spine in same person after leaving and then returning to the measuring table on the same day was less than 2% at each of the clinical sites. For most of the period of data collection, machines were cross-calibrated using a circulating Hologic anthropomorphic spine phantom, and each site maintained a quality assurance program.

Paragraph 6. A self-administered baseline questionnaire was used to obtain information about several other variables of interest. Demographic information included age and race/ethnicity. Women were asked their age when they first started competing for a running team and number of cross country seasons in which they had competed. They were asked to record the number of miles they ran per week during each competitive season (fall cross-country, winter track, spring track) and off-season (summer) in the previous year. From this information an average number of kilometers run per week was computed for the past year. Participants were asked what percentage of the distance was on pavement or concrete.

Paragraph 7. Women were asked to give a complete history of previous stress fractures that had occurred prior to baseline. They had to report confirmation by x-ray, bone scan, or magnetic resonance imaging for the stress fracture to be counted in these analyses. Eighteen women did not know whether they had experienced a previous stress fracture. These women are assumed not to have had a stress fracture in the analyses presented here.

Paragraph 8. Participants were asked to record their age at menarche and the number of menses they had in the previous 12 months. Women were classified as having current menstrual irregularity if they were oligomenorrheic (defined as 4-9 cycles in the past year) or amenorrheic (defined as fewer than 4 cycles in the past year). Women were also asked whether they had had 0, 1-3, 4-9, or 10-13 menses during each year after menarche. They were categorized as having a history of menstrual irregularity if they had ever been amenorrheic or oligomenorrheic since the year of menarche. Hormone concentrations were not measured, as this was beyond the scope of the study. A complete history of oral contraceptive use was obtained.

Paragraph 9. A modified version of the 97-item National Cancer Institute Health Habits and History food frequency questionnaire (8) was used to estimate usual nutrient intake during the previous six months. One of the modifications to the questionnaire was the inclusion of additional food items that were likely to be consumed by young athletes and that contained relatively high amounts of calcium. Only dietary calcium intake is included in the analyses presented in this paper. Use of calcium supplements tended to be

inconsistent and of short duration, and was not measured precisely enough for inclusion in these analyses. Three subscales (drive for thinness, bulimic tendencies, and body dissatisfaction) of the Eating Disorder Inventory (EDI) were used to identify subclinical eating disorders (3, 14, 15). A total EDI score was computed by summing the scores on each of the three EDI subscales. In the present study Cronbach's alpha for the three subscales was 0.79, indicating that the scores for the three subscales were to a large extent consistent with the total score.

Paragraph 10. Ascertainment of Stress Fracture Occurrence during Follow-up:

Participants were asked to record the occurrence of a possible stress fracture on a monthly calendar and also to report their occurrence to us immediately. The fracture had to be confirmed by x-ray, bone scan, or magnetic resonance imaging to be counted in this study. All reported stress fractures were in fact confirmed. The study paid for the imaging as needed. Participants were also queried periodically about the occurrence of stress fractures by e-mail, phone, and on their questionnaires. No additional stress fractures were reported as a result of these queries. No physical examinations were undertaken if a possible stress fracture was not reported.

Paragraph 11. Other Aspects of Follow-up: Participants were asked to return for bone densitometry and measurement of body composition, height, and weight one year and two years after baseline measurements. At this time they were asked to fill out a questionnaire covering most of the areas included at baseline, and every six months they filled out another food frequency questionnaire. This information is not, however, used

in the present analyses. Because the data were updated only at yearly intervals (or in the case of dietary intake at six month intervals), it was generally not possible to know whether any changes preceded or followed stress fracture occurrence, and therefore it was impossible to differentiate cause from effect.

Paragraph 12. In addition, in this young, mobile, and preoccupied population, not all participants had measurements made at the time requested. As mentioned above, no follow-up information at all was available for 23 (15%) of the original 150 women seen at baseline, and some were followed for less than two years. Baseline characteristics of those lost to follow-up were generally similar to those retained in the cohort, except that those lost to follow-up were more likely to have a history of stress fracture prior to baseline (52% vs. 32%, p=0.05). Among those who continued to participate in the study, we set a four-year limit as to how long we would wait for them to report for their follow-up visits. Only four runners had their final follow-up visit during the fourth year. Also, we included stress fractures that occurred up to one month after the final follow-up visit.

Paragraph 13. Statistical Analysis: Analyses were carried out with the SAS statistical package, version 8.02 (SAS Institute, Cary NC). Cox proportional hazards models were used to compute rate ratios for the rate of a first stress fracture during follow-up among those with a given characteristic divided by the rate of a first stress fracture during follow-up among those without the characteristic. Cox models were also used to estimate rate ratios according to the level of a characteristic and to compute rate ratios for one variable while controlling for the effects of other characteristics. Except for descriptive

information on the study population, all analyses controlled for clinical assessment site and group to which a participant was randomized. Additional control on actual oral contraceptive use during follow-up did not materially change any of the results. An examination of the degree of skewness of the variables indicated that none needed to be transformed.

RESULTS

Paragraph 14. The 127 participants were followed for stress fracture occurrence for a total of 2824 months, or an average of 1.85 years per woman. The age at baseline of the 127 runners ranged from 18 to 26 years, with a mean of 22.0 years. Table 1 provides other descriptive statistics on the cohort at baseline. About 83% were white, and their average body mass index was 21.2 kg/m^2 . Almost 31% reported having previously had one or more definite stress fractures, 57% had a history of menstrual irregularity, and 40% had previously used oral contraceptives.

Paragraph 15. Eighteen of the 127 runners had at least one stress fracture, for an average of 7.7 first stress fractures during the follow-up period per 100 person-years of follow-up. Ten of the first stress fractures occurred in the tibia, six in the foot, and two in the femur. Four runners had a second stress fracture: two in the tibia, one in the foot, and one in the femur.

Paragraph 16. Various factors were associated with elevated rate ratios for stress fracture during the follow-up period (Table 2). Women with a previous stress fracture had more than a five-fold higher rate of stress fracture during follow-up than women without such a history. Various indicators of lower bone mass were associated with an increased rate of stress fracture. For instance, for each standard deviation decrease (293.2 grams) in whole body bone mineral, the rate of stress fracture increased by almost twofold. Other factors associated ($p < 0.10$) with an increased rate of stress fracture were lower average daily dietary calcium intake and daily servings of dairy products, younger age at menarche, lower lean body mass, and lower weight. Younger age, shorter height, lack of previous oral contraceptive use, and a history of menstrual irregularity were also associated with increased rates of stress fracture, but these trends were not statistically significant. Little association was seen for current menstrual irregularity, percent body fat, BMI, age started running competitively, total competitive seasons run, kilometers run per week in past year, and total eating disorder inventory score from the three subscales.

Paragraph 16a. We attempted to examine whether previous stress fractures at certain sites were particularly strong predictors of stress fracture during follow-up. Numbers were small and confidence intervals very wide, but for the most common sites of previous stress fracture the point estimates of the rate ratio were quite similar: previous foot fracture 6.11 (2.11-17.68); previous tibia fracture: 7.91 (2.77-22.59); previous femur fracture: 6.78 (1.60-28.74); and previous fibula fracture: 6.98 (0.63-77.09).

Paragraph 17. We used a multivariate Cox model to identify variables that predicted stress fracture independently of the other variables under consideration. The various indicators of bone mass at different skeletal sites were highly correlated with each other, and we selected whole-body bone mineral content for our primary multivariate model because of the strength of its association with stress fracture, the multiple skeletal sites at which stress fractures can occur, and the limitations of using bone mineral density as a measure of bone mass, particularly when growth is still occurring (16). We subsequently present another model in which hip bone mineral density is used in place of whole-body bone mineral content because some readers will have a preference for that measure. Daily calcium intake and servings of dairy products were highly correlated ($r = 0.83$), and we chose dietary calcium intake for the multivariate model. Lean body mass was sufficiently highly correlated with bone mineral content that it could not be included in the same model. Accordingly, age, height, weight, history of stress fracture, age at menarche, history of menstrual irregularity, whole-body bone mineral content, and daily calcium in the diet were considered for inclusion in a multivariate model, along with certain other variables. Those significant at $p < 0.10$, and also a history of menstrual irregularity, for which the rate ratio was consistent with other studies even though $p > 0.10$, were included in the model presented here.

Paragraph 18. In the multivariate analysis (Table 3), a history of stress fracture was still a strong predictor of a future stress fracture, along with lower whole-body bone mineral content, decreasing age, younger age at menarche, and lower dietary calcium intake. A history of irregular periods was also associated with an increase rate of stress fracture,

although not statistically significantly so. Height, weight, BMI, percent body fat, age started running competitively, total competitive seasons run, kilometers run per week in past year, and total eating disorder inventory score did not predict stress fracture occurrence when entered into the multivariate analysis.

Paragraph 19. When hip bone mineral density was substituted for whole-body bone mineral content in the multivariate model, similar results were obtained (Table 4).

DISCUSSION

Paragraph 20. To our knowledge only one other study in runners, presented in abstract form (22), has examined whether a history of stress fracture predicts future stress fracture, and a positive association was found. A study in military recruits (24) reported an increase in risk that did not reach statistical significance. The rate ratio of 6.42 (1.80-22.87) associated with one or more previous stress fractures in the multivariate analysis here indicates that particular attention should be paid to this history, as these individuals appear to be at especially high risk of additional stress fractures. Our results also indicate that a history of stress fractures is a marker of susceptibility above and beyond its association with bone mineral content or density and the other variables included in the multivariate analyses. Runners and their coaches should be made aware of the high risk for additional fractures, should try to identify the reason for the high risk, and make changes so as to reduce that risk.

Paragraph 21. Our finding that lower bone mass is associated with an increased risk for stress fracture is consistent with other prospective studies carried out in competitive athletes (6, 22) and military recruits (19). Thus, it is likely that lower bone mass is indeed predictive.

Paragraph 22. Several previous studies in competitive athletes and military recruits have reported that current or past menstrual irregularity is associated with an increased risk for stress fracture (2, 6, 7, 9, 21, 22, 24). Our rate ratio of 3.41 (0.69-16.91), although not statistically significant, is consistent with these other reports. Studies not finding this association had very small numbers of amenorrheic participants (19) or had participants with only short periods of amenorrhea (10). Menstrual irregularities often occur in association with low serum estrogen concentrations and are known to be related to low bone mineral density and low serum concentrations of bone formation markers (12, 20, 23, 30). The results of our multivariate analysis suggest that a history of menstrual irregularity may have additional adverse effects on bone health beyond its associations with lower bone mineral content and density. Efforts should be made to identify reasons for the menstrual irregularities, such as inadequate diet, and appropriate changes made.

Paragraph 23. Low calcium and dairy product intake has been associated with decreased bone mineral density in young adult women (25). One previous study in competitive athletes found lower calcium and dairy product intake to be associated with an increased risk for stress fracture (21). Also, a report available in abstract form (18) from a recent randomized trial of supplementation with 2000 mg calcium and 800 International Units

(IU) of Vitamin D among female Navy recruits in basic training, found that in just eight weeks the supplemented group had a 27% lower incidence of stress fracture than the non-supplemented group, using a per protocol statistical analysis. On the other hand, another observational study in track and field athletes (6) and other observational studies in military recruits (10, 19) have not shown any protective effect. Among the studies showing no effect, the prospective study of Bennell et al. (6) reported that most of the track and field athletes had high intakes of dietary calcium, and were thereby possibly already receiving whatever protection dietary calcium provides against stress fracture. The questionnaire used in another study (19) assessed only whether the recruits had at least one serving of milk, cheese, or yogurt per day, and thus did not attempt to collect detailed quantitative information on calcium intake. The other study (10) asked soldiers to recall diet during adolescence, and errors in recall would have been likely. Thus, uncertainty remains about the role of lower calcium intake on stress fracture occurrence. In our study lower dietary calcium and dairy product intake were associated with an increased risk of stress fracture independently of their association with bone mineral content or density. Some other aspect of bone strength may be affected by calcium intake as well. For instance, insufficient dietary calcium would also be expected to result in inadequate repair of microdamage (21) or may have a detrimental effect on some aspect of bone geometry, such as cortical thickness (26), and thereby increase the risk for stress fractures.

Paragraph 23a. Increasing calcium intake in those consuming inadequate amounts would be a relatively easy preventive measure to implement, so determining its importance with

more certainty is of high priority. More research should be undertaken to determine the optimal level of calcium intake of distance runners and of athletes in general. The protection indicated by the randomized trial of 2000 mg per day of calcium with 800 IU per day of Vitamin D in Navy recruits (18) might indicate that among highly active young women such as military recruits and competitive distance runners, higher levels of calcium intake are needed than the recommended dietary allowance of 1300 mg/day for those of ages 9-18 years and 1000 mg/day for those of age 19 years and older in the general population.

Paragraph 24. Whether increasing age is associated with a greater risk, a reduced risk, or no change in risk for stress fracture has been controversial (4). Across the age range of 18-26 years considered in this study, it would be expected that younger runners would have higher stress fracture rates because bone mass is still gained through the third decade of life (25). It should also be noted that the decreasing stress fracture rate with increasing age in the present study was seen only in the multivariate analysis when we accounted for a history of stress fracture.

Paragraph 25. Other studies of athletes in this age group (4, 6, 7, 9, 21) have found either a positive association between age at menarche and stress fracture risk or no association. In contrast, we found that younger age at menarche was associated with a higher rate of stress fracture. Most studies, but by no means all (reviewed in 4, 13), have found that age at menarche is inversely correlated with bone mineral density and bone mineral content, but in the present study we found that age at menarche had only a slight inverse

correlation with whole-body bone mineral density ($r=-0.13$, $p=0.12$), but no correlation with whole-body bone mineral content ($r=0.03$, $p=0.76$). If later age at menarche results in later maturation and consolidation of bone, one would expect higher rates of stress fracture with later age at menarche, as reported by others. It is possible that some other aspect of bone strength associated with late age at menarche is playing a role in the decreased risk found in our study. Among the determinants of bone strength are bone size, cortical thickness and porosity, the number of trabeculae, trabecular thickness and connectedness, tissue mineral content, the presence of microfractures, and the direction and extent of cross-linking of collagen (28). Further studies are needed before any definitive conclusions are reached.

Paragraph 26. Although we previously reported an association at baseline between disordered eating and low bone mineral density among eumenorrheic runners (11), no association between disordered eating and subsequent stress fracture occurrence was seen in these analyses. Numbers of stress fractures, however, were too small to consider the rates of stress fracture by menstrual status and eating disorder status simultaneously.

Paragraph 27. Finally, we did not find training-related factors to be important, including age started running competitively, total competitive seasons run, miles run per week in past year, and miles run on concrete or pavement. The number of stress fractures was too small to enable us to examine these factors in detail, but the results of the present study are consistent with those reported by others (6, 21). Although it does not appear that training-related factors are important in the etiology of stress fracture at least among

athletes who have been participating in their sport for several years, more study with larger numbers of stress fractures and with more variation in length and type of training is needed before definitive conclusions are reached.

Paragraph 28. Our prospective study had the advantage of collecting information on possible risk factors before the occurrence of the stress fractures, thus eliminating the possibility of biased recall once a stress fracture has occurred. In addition, all participants were from one sport, cross-country running, thus eliminating sport as a potential source of variation. On the other hand, our study population was of modest size, and the number of stress fractures was only 18. Accordingly, we could not identify small increases or decreases in risk. Because of limited resources, physical examinations were not conducted on those who did not report possible stress fractures, and measurements of serum hormone concentrations were not made on any participants. Also, despite our best efforts, we had no follow-up information on 15% of the original participants. We found this age group, with its high degree of mobility and changing interests over time, to be particularly challenging to retain in a longitudinal study. Because the major objective was to conduct a randomized trial of the effect of oral contraceptives, we did not collect information on a wide spectrum of possible risk factors. In addition, it should be emphasized that we did focus on only one sport. None of the risk factors identified is specific to cross-country track, and evaluating these risk factors in athletes in other sports could give wider applicability to our findings.

Paragraph 29. In conclusion, the results of our study and those of others indicate that young female runners with previous stress fractures, lower bone mass, and a history of irregular menstrual periods are at high risk for stress fracture and should be carefully monitored. Although the evidence is not definitive, high calcium intake should be encouraged. The relation between age at menarche and risk for stress fracture is unclear, and needs further study.

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TABLE 1. Mean \pm one standard deviation or percentage with selected characteristic at baseline

Characteristic	Mean \pm 1 Standard Deviation or Percentage
Age (years)	22.0 ± 2.6
Height (cm)	165.9 ± 6.1
Weight (kg)	58.3 ± 6.7
Body mass index (kg/m^2)	21.2 ± 1.9
Percent body fat	$23.0 \pm 5.3\%$
Race/ethnicity	
White	83.5%
Hispanic	3.9%
Asian/Pacific Islander	8.7%
Black	0.8%
Other	3.1%
Age started running competitively (years)	14.2 ± 3.5
Total number seasons run competitively	11.9 ± 6.8
Average distance run per week, past year (km)	55.5 ± 18.0
Percent of distance on pavement or concrete	$65.6 \pm 22.1\%$

History of one or more stress fractures	30.7%
Age at menarche (years)	13.1 ± 1.5
History of menstrual irregularity*	57.1%
Menstrual irregularity (past year)†	33.1%
Ever used oral contraceptives	39.7%
Total eating disorder inventory score‡	11.8 ± 12.4
Whole-body bone mineral content (g)	2169.3 ± 293.2
Bone mineral density (g/cm ²)	
Hip	0.986 ± 0.116
Spine	0.988 ± 0.108
Whole body	1.111 ± 0.084
Daily dietary calcium intake (mg)	1357.5 ± 681.4

* ≤ 9 menstrual periods in any year, excluding the year of menarche.

† ≤ 9 menstrual periods in the year before baseline.

‡ Total eating disorder inventory score, which can range from 0-69, is the sum of the scores from three subscales. See Garner and Olmstead (14).

TABLE 2. Adjusted* rate ratios (and 95% confidence interval) for associations between selected characteristics and stress fracture

Characteristic	Rate Ratio (95% CI)
Age (per year younger)	1.12 (0.89,1.41)
Height (per cm shorter)	1.04 (0.96, 1.12)
Weight (per kg decrease)	1.08 (0.99, 1.16)
Body mass index (per kg/m ² decrease)	1.20 (0.90, 1.61)
Percent body fat (per 5 % increase)	1.16 (0.71, 1.89)
Lean body mass (per kg decrease)	1.14 (1.01, 1.28)
Age started running competitively (per year younger)	1.01 (0.93, 1.10)
Total number competitive seasons (per season)	1.01 (0.93, 1.10)
Average distance run per week, past year (per 10 km increase)	1.08 (0.81, 1.45)
Percent distance on pavement or concrete (per 5% decrease)	1.05 (0.94, 1.18)
History of one or more stress fractures (yes/no)	5.24 (1.88, 14.49)
Number of previous stress fractures (per each previous fracture)	1.59 (1.15, 2.19)
Age at menarche (per year younger)	1.37 (0.97, 1.92)
History of menstrual irregularity† (yes/no)	1.90 (0.66, 5.51)

Menstrual irregularity in past year‡ (yes/no)	1.05 (0.38, 2.89)
Never used oral contraceptives (yes/no)	2.22 (0.65, 7.69)
Total eating disorder inventory score§ (per 5 units)	1.03 (0.86, 1.24)
Whole-body bone mineral content (per standard deviation decrease, where 1 standard deviation = 293.2 g)	1.79 (1.02, 3.13)
Hip bone mineral content (per standard deviation decrease, where 1 standard deviation = 5.78 g)	1.69 (0.95, 2.94)
Spine bone mineral density (per standard deviation decrease, where 1 standard deviation = 0.11 g/cm ²)	1.89 (1.04, 3.45)
Hip bone mineral density (per standard deviation decrease, where 1 standard deviation = 0.12 g/cm ²)	1.45 (0.81, 2.56)
Whole body skeletal area (per standard deviation decrease, where 1 standard deviation = 166.8 cm ²)	1.89 (1.06, 3.33)
Daily dietary calcium intake (per 100 mg decrease)	1.08 (0.99, 1.18)
Daily servings of dairy products (per one serving decrease)	1.41 (1.01, 1.96)

*Adjusted by Cox proportional hazards model for clinical site and treatment group assignment.

†≤ 9 menstrual periods in any year, excluding the year of menarche.

‡≤ 9 menstrual periods in the year before baseline.

§ Total eating disorder inventory score, which can range from 0-69, is the sum of the scores from three subscales. See Garner and Olmstead (14).

TABLE 3. Multivariate adjusted* rate ratios (and 95% confidence intervals) for associations between selected characteristics and stress fracture, whole body bone mineral content used as measure of bone mass

Characteristic	Rate Ratio (95% CI)
Age (per year younger)	1.42 (1.05, 1.92)
History of one or more stress fractures (yes/no)	6.42 (1.80, 22.87)
Whole-body bone mineral content (per standard deviation decrease, where 1 standard deviation = 293.2 g)	2.70 (1.26, 5.88)
Daily dietary calcium intake (per 100 mg decrease)	1.11 (0.98, 1.25)
Age at menarche (per year younger)	1.92 (1.15, 3.23)
History of menstrual irregularity† (yes/no)	3.41 (0.69, 16.91)

*Adjusted by Cox proportional hazards model for clinical site, treatment group assignment, and all the other variables in the table.

†≤ 9 menstrual periods in any year, excluding the year of menarche.

TABLE 4. Multivariate adjusted* rate ratios (and 95% confidence intervals) for associations between selected characteristics and stress fracture, hip bone mineral density used as measure of bone mass

Characteristic	Rate Ratio (95% CI)
Age (per year younger)	1.42 (1.03, 1.95)
History of one or more stress fractures (yes/no)	6.71 (1.93, 23.35)
Hip bone mineral density (per standard deviation decrease, where 1 standard deviation = 0.12 g/cm ²)	2.16 (1.04, 4.48)
Daily dietary calcium intake (per 100 mg decrease)	1.09 (0.97, 1.23)
Age at menarche (per year decrease)	1.61 (1.04, 2.49)
History of menstrual irregularity† (yes/no)	3.10 (0.70, 13.74)

*Adjusted by Cox proportional hazards model for clinical site, treatment group assignment, and all the other variables in the table.

†≤ 9 menstrual periods in any year, excluding the year of menarche.

**The Effect of Oral Contraceptives on Body Weight and Body Composition in Young
Female Runners, Elizabeth Procter-Gray,...*et al.***

ABSTRACT

Purpose: To determine the effect of oral contraceptives (OCs) on body weight, fat mass, percent body fat, and lean mass in young female distance runners. **Methods:** The study population consisted of 150 female competitive distance runners aged 18-26 years who had participated in a randomized trial of the effect of oral contraceptives (30 µg ethinyl estradiol and 0.3 mg norgestrel) on bone health for an intended two years. As part of this trial, weight and body composition were measured approximately yearly by balance-beam scales and dual energy x-ray absorptiometry, respectively. **Results:** Women assigned to OCs gained slightly less weight than control women (adjusted mean difference (AMD) = -0.54 ± 0.31 kg/year, $p=0.09$) and tended to gain less fat (AMD = -349.1 ± 250.0 g/year, $p=0.16$), regardless of baseline menstrual status. OC assignment was associated with a significant gain in lean mass relative to controls among eumenorrheic women (AMD = 771.8 ± 172.3 g/year, $p<0.0001$), but not among women with fewer than 10 menstrual cycles in the year prior to baseline (AMD = 16.2 ± 346.7 g/year, $p=0.96$). Treatment-received analyses yielded similar results. **Conclusion:** The results of this randomized trial confirm previous findings that OCs do not cause weight or fat gain, at least among young female runners. Further study is needed to evaluate our finding that OC use is associated with lean mass gain in eumenorrheic, but not in amenorrheic and oligomenorrheic, runners. **Key words:** RANDOMIZED TRIAL; WEIGHT; FAT; LEAN MASS; LONG DISTANCE RUNNERS

INTRODUCTION

Since the U.S. Food and Drug Administration (FDA) approval of the contraceptive pill in 1960, its popularity among women has attested to its general effectiveness and tolerability. According to Centers for Disease Control and Prevention, 11.6 million women used oral contraceptives (OCs) in the United States in 2002, making it the most common method of birth control.¹ However, questions remain among scientists, health care providers, and users regarding possible side effects.

Especially among young women, fear of weight gain (or body fat gain) has been cited as a primary reason for avoiding oral contraceptive use.^{2,3,4,5,6} Many studies have investigated the oral contraceptive-weight gain connection, and results have varied from a positive OC-weight gain association^{7,8} to no association.^{9,10,11,12,13,14} In a review of 42 randomized controlled trials, Gallo et al.¹⁵ concluded that the evidence is insufficient to rule out an association, but no large effect is evident, and no dose-response relationship is observed between estrogen use and weight gain. Gallo et al. described several mechanisms that have been hypothesized to account for weight gain with OCs, including fluid retention, increased subcutaneous fat, and anabolic or psychological effects on appetite. Further investigation of this matter is warranted, as it may affect the decision-making of teenagers and young women in need of a reliable form of birth control. Also, any association between OCs and body composition may be of special interest among the population of female runners, as it may affect their running performance.

This paper investigates the changes in weight and body composition in female competitive runners who were recruited for a randomized trial designed primarily to

assess the effect of oral contraceptives on bone mineral density and stress fracture incidence.

METHODS

Participants and recruitment

Details of the study methodology have been described by Cobb et al.¹⁶ One hundred fifty competitive female runners were recruited between August 1998 and September 2003 from inter-collegiate cross country teams, post-collegiate running clubs, and road races mainly in the geographic areas of Palo Alto, CA, Los Angeles, CA, Ann Arbor, MI, West Haverstraw, NY, and Boston, MA. To be eligible, women had to be 18-26 years old, run at least 40 miles/week during peak training times, and compete in running races. Exclusion criteria included medical contraindications to OC use, unwillingness to be randomized to OC use or non-use, and any use of OCs or other hormonal contraception within the past six months. All women were required to visit a study physician or student health service staff member prior to enrollment to rule out contraindications to OC use. Written informed consent was obtained from each subject after she received a detailed explanation of the study procedures. The protocol was approved by the Institutional Review Boards of the U.S. Army Medical Research and Materiel Command, the colleges from which participants were recruited, and the clinical sites: Stanford University, the University of California Los Angeles, the University of Michigan, the Helen Hayes Hospital, and the Massachusetts General Hospital.

Randomization and intervention

Eligible women were randomly assigned to receive OCs or no intervention for an intended 2 years, stratified according to clinical site. An independent investigator who was not otherwise affiliated with the study performed the randomization using a random number table. The OC pill assigned in this study was Lo/Ovral (Wyeth Ayerst, 28-day pack), which contains 30 µg ethinyl estradiol and 0.3 mg norgestrel. For ethical reasons, neither the athletes nor prescribing physicians were blinded to treatment assignment, and no placebo was used.

Data collection and follow-up

At baseline, participants visited one of the clinical sites for measurement of bone mineral density, body composition, height, and weight. Height and weight were measured using standard stadiometers and balance-beam scales, respectively. Body composition (fat mass, percent body fat, and lean mass) was measured by dual energy x-ray absorptiometry (DXA; QDR 4500A, Hologic). All women were asked to refrain from heavy physical activity 24 hours prior to measurement in order to minimize fluctuations in hydration level.

Participants also filled out questionnaires on menstrual history, previous use of OCs, injury and stress fracture history, training regimen, diet, eating attitudes, and eating behaviors, as previously described.¹⁷ Women were classified as amenorrheic, oligomenorrheic, or eumenorrheic based on the number of menses they reported having in the previous 12 months. Amenorrhea was defined as 0-3 cycles in the past year; oligomenorrhea as 4-9 cycles in the past year; and eumenorrhea as 10 or more cycles in

the past year. Participants were asked to return one year and two years later to repeat these measurements and questionnaires.

Of the 150 women randomized, 124 (83%) attended at least one follow-up appointment and 96 (64%) participants attended both, at an average of 14.4 months (median: 13.1 months) and 26.6 months (median: 25.4 months), respectively, after baseline. Baseline characteristics of the participants with no follow-up data were similar to those with follow-up data, except that those with no follow-up were more likely to have a history of stress fracture prior to baseline (52% vs. 32%, p=0.05). Between clinic visits, participants filled out a monthly calendar on which they recorded menstrual bleeding and the use of OC pills.

Ascertainment of compliance

Women in the treatment group were asked to report if and when they discontinued taking the study medication. Treatment compliance was also monitored through return of used pill packs, monthly calendars, and yearly questionnaires. If a woman reported having discontinued treatment, she was contacted by a research assistant to determine if and when OCs were discontinued and the reason why. Similarly, women in the control group were asked to contact us if they were planning to start an OC. If so, they were encouraged to take the study pill (Lo/Ovral) or a pill with a similar dose of estrogen. Compliance was also monitored on monthly calendars and yearly questionnaires. If a woman reported having started OCs, she was contacted by a research assistant to obtain the date of starting OCs, as well as the formulation and the reason for starting them.

Statistical design and analysis

The study was powered to detect differences in bone mineral density and stress fracture occurrence between the OC group and control group, as these were the outcomes of the primary study, rather than to detect differences in rates of change in weight or body composition. Statistical analyses were performed using the SAS statistical package, version 8.2 (SAS Institute, Cary, NC, U.S.A.). For preliminary descriptive analyses, means were compared between groups using t-tests for normally distributed variables and Wilcoxon sum-rank tests for non-normally distributed variables. Proportions were compared using chi-square tests or Fisher's exact test, in the case of small numbers in cells.

The intention-to-treat method was used for the primary analyses described here. The primary outcomes examined were rates of change in (1) weight, (2) body mass index (BMI), (3) fat mass, (4) body fat as percentage of total mass, and (5) lean mass. Linear mixed-effects models were used to evaluate the effect of OC assignment on each outcome. Results for BMI change were identical to results for weight change, since the women's heights were constant. BMI is not discussed further. Multiple regression models for within-woman rate of change (last measurement minus baseline divided by follow-up time) were used to confirm the mixed-model results, and a sensitivity analysis was performed to compare various ways of handling missing data.¹⁸ In this analysis the linear regression models were run using data from (1) only those subjects who attended the second follow-up exam ("completers only"), (2) subjects who had at least one follow-up exam, (3) all women enrolled in the study, with last body composition measurements

carried forward, (4) all women enrolled, with the mean change imputed for those missing the second follow-up, and (5) all women enrolled, with first observation carried forward for those missing the second follow-up. All variations of the analysis produced results similar in magnitude and statistical significance to the results reported here, and they are therefore not presented.

To reflect study design, clinical site was included as a covariate in all models. Additionally, variables that differed ($p < 0.10$) between the OC and control groups in baseline comparisons, along with menstrual status at baseline and eating disorder inventory score¹⁹ as *a priori* variables of interest, were tested for an interaction effect or confounding effect (>15% change in parameter) on the association of interest, and, if present, included in the model. Because of the small number of women in the amenorrheic category, we combined amenorrheic and oligomenorrheic women into one category, “irregular” (0-9 menses in the past year) and contrasted them with eumenorrheic women, termed “regular” (10 or more menses in past year).

Secondary, treatment-received analyses evaluated the effect of the actual proportion of follow-up time that the women took oral contraceptives (regardless of treatment assignment) on weight and body composition. Each woman was assigned a proportion score ranging from 0 to 1, with 1 representing the use of OCs for 100% of the follow-up period, 0.5 representing 50%, and so forth. We performed other treatment-received analyses, classifying women into the OC group if they used OCs for (1) three months or more, and (2) six months or more. Linear mixed-effects models, with the same covariates and stratification used as in the intention-to-treat analyses, were used to evaluate the differences between outcomes for OC users and non-users. Findings from

these analyses are presented in the Results section following the intention-to-treat analysis. We compared ≥6-month OC users vs. <6-month users in baseline and follow-up characteristics in order to identify any potential confounders in the treatment-received analysis.

RESULTS

Primary analysis

Baseline characteristics

At baseline, the mean age for all 150 women was 22.1 years, mean height was 165.6 cm, and weight was 58.2 kg, with a mean BMI of 21.1 kg·m⁻². Mean caloric intake was 2278 kcal/day. The women ran for an average of 56.0 km/week, and lifted weights 66.5 minutes/week. Fifty participants (33%) reported irregular menses in the past year.

Upon randomization, 69 women (46%) were assigned to the OC group and 81 (54%) to the control group (discrepancy due to chance). The two groups were very similar at baseline in the four measurements of interest to this analysis: weight, fat mass, percent fat, and lean mass. The women were also well-matched in mean age, height, caloric intake, total number of previous menstrual periods, and several other physiological and behavioral characteristics (Table 1). However, women assigned to OCs had dieted more frequently and tended to score higher on the eating disorder inventory than control women.

Retention and adherence

Twenty-six women (17%) withdrew from the study or provided no further body composition data after baseline (Figure 1). This left 124 women (54 (78%) of the OC

group and 70 (86%) of the control group) with at least one follow-up visit for the weight analysis. Lean mass, fat mass, and percent fat were not obtained from one additional control woman.

Within the treatment group, 14 (26%) stopped taking OCs after an average of 5.4 months, and 28 (40%) of the control group started taking them at an average of 11.3 months into the study. Four women in the control group and one woman in the treatment group switched groups twice. The reasons women gave for stopping OCs included (in decreasing order of frequency): fear of weight gain or perceived weight gain, side effects (irritability, abdominal symptoms, nausea, fatigue, or unspecified), and fear of detriment to athletic performance. The reasons control women gave for starting OCs included (in decreasing order of frequency): to regulate periods, to alleviate menstrual symptoms and cramps, to prevent pregnancy, to treat acne, and to treat allergies.

Women who stopped taking OCs had a lower percentage of body fat, fewer menstrual periods, and more disordered eating than those who continued to follow the OC protocol. Amenorrheic women were the least likely to comply with taking OCs. Of eight amenorrheic women who were assigned to OCs, only one took them through the entire study. Of the remaining seven, two were lost to follow-up, five discontinued OCs within two months, and one discontinued OCs after 1.5 years.

Body composition changes by treatment randomization (intention-to-treat analysis)

(1) Change in weight. The weight of women assigned to OCs did not increase relative to controls. In fact, controlling for clinical site, baseline menstrual status, and baseline dieting frequency in linear mixed models analysis, assignment to OCs was associated with a trend toward lower weight gain ($p=0.09$, Table 2). Trends were similar

regardless of baseline menstrual status, eating disorder inventory score and baseline weight.

Four women gained or lost over 10 kg during the course of the study. Removal of these women from the analysis resulted in a greater weight gain among controls relative to the OC group and thus only strengthens the observation that OC assignment did not contribute to weight gain.

(2) Change in fat mass and body fat percentage. Women assigned to OCs did not show an increase in fat mass or in percent body fat relative to controls. Mixed effects models controlling for clinical site and baseline menstrual status showed a trend toward relative fat loss associated with OC assignment ($p=0.16$, Table 2). Results were similar when the analysis was stratified by baseline menstrual category or eating disorder score. For percentage body fat, there was again a trend toward relative fat loss in those assigned to OCs relative to controls ($p=0.08$, Table 2).

(3) Change in lean mass. The OC-lean mass association differed according to baseline menstrual status. Assignment to OCs was associated with significantly greater lean mass gain relative to controls among women with regular menses ($p<0.0001$, Table 2), but there was no association among those with irregular menses ($p=.01$ for the effect modification by menstrual regularity). Eumenorrheic women assigned to OCs had an adjusted mean gain of 675.5 ± 154.3 g/year while controls showed little change in lean mass (-96.3 ± 137.5 g/year). Women who had irregular menstrual cycles at baseline gained, on average, moderate amounts of lean mass regardless of treatment assignment. Omission of the four most extreme values for lean mass change did not affect the above results.

To preserve the benefits of random assignment, characteristics that differed between the two groups after randomization were not included as covariates in the primary intention-to-treat analyses. However, women assigned to OCs differed from controls by the second follow-up visit in three respects: a higher percentage of fat in the diet, more frequent weight lifting, and fewer kilometers run per week than control women (data not shown). When the measure of weight lifting was added to the mixed effects models above, the adjusted mean difference for eumenorrheic women fell by 15%, but the OC-lean mass association remained positive and statistically significant ($p=0.0004$), Addition of other follow-up variables did not change the magnitude or significance of the outcome.

Secondary analysis

Actual use of oral contraceptives

Among the 124 women with follow-up, 75 took OCs for three months or more, and 59 used them for six months or more, ignoring treatment assignment

Baseline and follow-up characteristics

Women who used OCs for six months or more differed from those who did not in a few baseline characteristics (data not shown). Those who had used OCs for at least six months had lower mean bone mineral content. They less frequently reported a history of menstrual irregularity and current menstrual irregularity at baseline. However, the two groups did not differ in number of total lifetime menstrual periods.

Follow-up measures of menstrual cycles, diet, and exercise characteristics revealed that the women who took OCs for at least six months had predictably regular periods (having a greater number of menses per year than non-OC users), but the only other difference between the groups was that OC users had a higher percentage of fat in the diet at the second follow-up (data not shown).

Body composition changes by actual use of OCs (treatment-received analysis)

(1) Change in weight. As in the intention-to-treat analysis, there was little association between the proportion of follow-up time on OCs and the rate of change in weight (Table 3). Adding covariates from the set of variables that differed between OC users and non-users did not change the results, so we did not include these covariates in the models presented here. When women were classified by use of OCs for (1) three months or more, and (2) six months or more, comparisons to the non-OC group again revealed little difference in weight change (Table 3). In all analyses, the OC users gained, on average, slightly less weight than non-users.

(2) Change in fat mass and body fat percentage. There was no association between body fat change and actual OC use in the proportionate, 3-month-use, or 6-month-use analysis (Table 3).

(3) Change in lean mass. As in the intention-to-treat analysis, women with regular menses at baseline showed a significant relative increase in lean mass associated with the proportion of time that they were actually taking OCs ($p<0.0001$, Table 3). The association with OC use was also seen in the analyses of 3-month users ($p=0.005$) and 6-

month users ($p=0.0007$). No association between actual OC use and lean mass change was found among women with irregular menses (Table 3).

Because weight lifting could have a strong effect on lean mass, we explored adding it to the models above, even though the two treatment-received groups did not differ significantly in their frequencies of weight lifting ($p=0.14$). The adjusted mean differences fell by 15 – 32%, but the OC-lean mass association remained positive and statistically significant in the proportionate treatment-received analysis ($p=0.002$) and in the 6-month treatment-received analysis ($p=0.01$). The association was of borderline significant in the 3-month-use analysis ($p=0.06$).

DISCUSSION

Our finding of little difference in weight gain or fat gain between women assigned to 30 μg ethinyl estradiol plus 0.3 mg norgestrel versus no treatment adds further confirmation to the conclusion reached in the review by Gallo et al.²⁰ that no large positive effect of OC use on weight is likely. That we found a (non-significant) trend toward less weight and fat gain among OC users as compared with non-users should reassure athletes that OCs will not cause performance-impairing weight changes. The results of our treatment-received analyses bolster our confidence in this conclusion. Because of this study's focus on young female runners, we cannot rule out an effect of OC use for inactive women or for those outside the 18-26 year age range.

We confirm others' findings²¹ that weight gain is still commonly believed to be a side effect of OC use despite evidence to the contrary. Fear of or perceived weight gain was the most common reason given (cited by 6 of 14) for discontinuing OCs in our study.

Although we do not have weight measurements from women at the point of stopping OCs, if we compare the weight change of switchers in the first year to that of control women, the difference is insignificant (0.4 kg/year for switchers vs. 0.1 kg/year for controls, p=0.56). Our study should re-assure women that OCs are not a cause of weight gain.

The unanticipated positive association between OC use and lean mass gain observed among eumenorrheic women merits further investigation. The association was strongest in the intention-to-treat analysis, and was also present, but slightly weaker, in the treatment-received analysis. There are few other reports with which to compare our findings. Machado et al²² found that women randomized to estradiol-gestodone for one cycle only showed a significant increase in fat-free mass relative to controls. Women assigned to estradiol-drospirenone did not.

Several previous studies^{23, 24, 25, 26, 27} have investigated the lean mass/muscle strength association with hormone replacement therapy (HRT) in postmenopausal women. The results of these trials have been inconsistent, however, and caution is required in extrapolating results to young women on OCs.

If there is an anabolic effect of combination OCs on lean mass, the mechanism remains unclear. Friend et al.²⁸ proposed that estrogen may have an anabolic action by increasing serum growth hormone concentrations. Skeletal muscle contains some estrogen receptors and thus may be affected directly as well. Phillips et al.²⁹ demonstrated measurable increases in muscular force of the adductor pollicis when estrogen levels rose in the follicular phase of young women's menstrual cycles. In contrast, multiple studies have found that OCs are associated with decreased free

testosterone and DHEAS in healthy women of reproductive age,³⁰ potentially causing a decrease in lean mass relative to controls.

It is impossible to tell whether the estrogen and/or progestin component of the OC used in this trial might be associated with the lean mass increase observed. The progestin component of Lo/Ovral, Norgestrel, has the highest androgenic activity of the four first-generation progestins investigated in a 2006 US Pharmacist report,³¹ and so could have a muscle-building effect.

It is unclear why, as in our results, a stimulatory effect of OCs on lean mass would be observed in eumenorrheic women but not in women with irregular menstruation. Could there be some threshold estrogen level below which the OCs do not have a stimulatory effect? Alternatively, irregular periods could be a marker for caloric deprivation. Ihle and Loucks³² found that caloric deprivation promoted bone resorption and interfered with bone formation, and Frost³³ proposed that the “mechanostat” that adjusts bone to the mechanical stresses put upon it might be reset by energy availability or hormone levels. Similar mechanisms could apply to muscle mass. Could the women with irregular periods in this study have a dietary deficit that prevents them from putting on the lean mass that Lo/Ovral might normally stimulate? Self-reported calories did not differ between menstrual groups at baseline, but women with irregular periods had a lower percentage of fat in the diet.³⁴ This difference disappeared, however, at the first and second follow-up visits (data not shown).

Our study confirms the difficulty of conducting a randomized controlled trial with oral contraceptives, which so greatly affect the personal lives of participants. Non-compliance was high in both treatment and control groups. We found women in the age

range 18-26 years to be a difficult group to follow in a study of this length because of their high mobility and multiple interests. We were unable to obtain any follow-up weight and body composition measurements on 17% of the subjects, and one-third were lost from the study by the end of the follow-up period.

For ethical reasons, women were not blinded regarding their use or non-use of OCs, and this could have introduced bias into the study, especially given the widespread belief among young women that OC use leads to weight gain. It is possible that the women assigned to OCs compensated for that assignment in some way, for example, by cutting back on calories or increasing their activity. As noted above, by the second follow-up visit the OC group had a higher mean frequency of weight training, but they had a lower mean number of kilometers run per week, and their self-reported calorie consumption was indistinguishable from that of controls. While self-reported dietary logs are notoriously inexact, there is little reason to believe that errors in reporting would be differentially erroneous between the two groups in this study.

Because this project was initially designed to assess bone mineral density and stress fracture as primary outcomes (not weight change), we did not maintain standardized conditions at the weigh-in regarding clothing worn, time of day, or day in menstrual cycle, and this could have introduced some inexactness of measurement.

Despite these weaknesses, the randomized design of this trial, its assessment of a great number of lifestyle variables, and its duration should lend confidence to our conclusions that young female distance runners can take oral contraceptives without fear of resultant weight or fat gain. Evaluation in other studies of the effect of OCs on lean mass may clarify whether OCs can enhance muscle strength.

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PLEASE NOTE: REFERENCES SHOULD BE PLACED BEFORE THE FIGURE AND TABLES. I TRIED TO PUT A PAGE BREAK AFTER THE REFERENCES, AND WORD DIDN'T SEEM TO ALLOW IT. I'LL SEND THIS ON AS IS AND TRY TO TINKER BEFORE SUBMITTING FOR PUBLICATION.

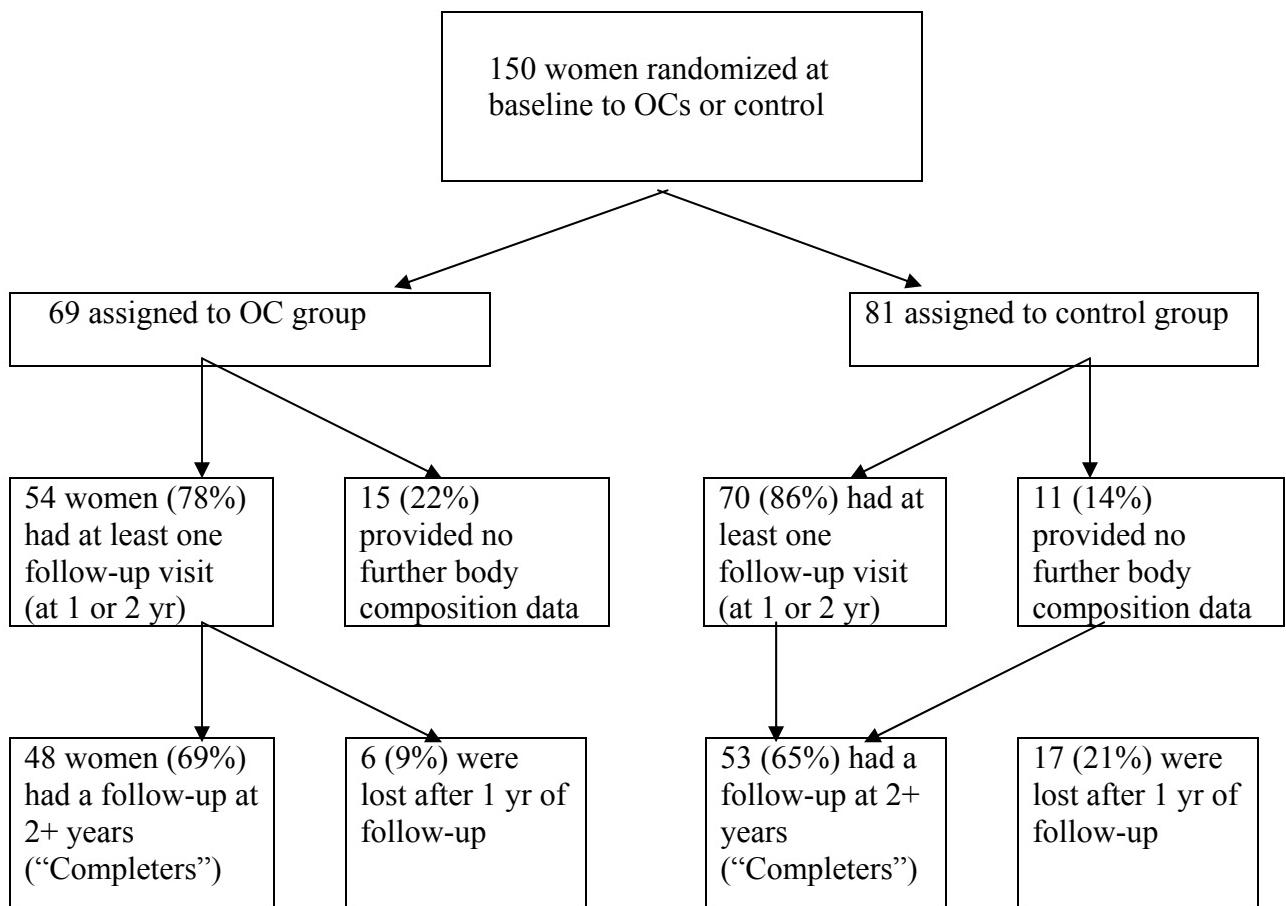


Figure 1. Participation and follow-up.

TABLE 1. Baseline characteristics by treatment assignment: mean \pm standard deviation or percentage

	<u>Treatment Assignment</u>	
	Oral contraceptives (N=69)	Control (N=81)
Weight (kg)	58.2 \pm 7.3	58.1 \pm 6.6
Fat mass (kg)	13.1 \pm 4.0	13.5 \pm 4.1
Percent body fat	22.7 \pm 5.2	23.3 \pm 5.4
Lean mass (kg)	41.8 \pm 4.9	41.7 \pm 4.2
Age (yr)	22.3 \pm 2.7	21.9 \pm 2.6
Height (cm)	165.9 \pm 6.6	165.4 \pm 6.1
Body mass index ($\text{kg}\cdot\text{m}^{-2}$)	21.1 \pm 1.9	21.3 \pm 2.0
Caloric intake ($\text{kcal}\cdot\text{day}^{-1}$)	2250 \pm 894	2302 \pm 988
Percent of calories from fat	17.6 \pm 6.5	18.4 \pm 6.5
Protein intake ($\text{g}\cdot\text{day}^{-1}$)	92.9 \pm 41.4	91.6 \pm 39.9
Dietary calcium ($\text{mg}\cdot\text{day}^{-1}$)	1394 \pm 830	1412 \pm 670
Frequency of dieting in past year*†	2.0 \pm 1.3	1.5 \pm 0.8
Total EDI score‡•	14.7 \pm 14.7	10.6 \pm 11.8
Distance run in past year ($\text{km}\cdot\text{wk}^{-1}$)	56.0 \pm 16.9	56.0 \pm 18.3
Time lifting weights in past year ($\text{min}\cdot\text{wk}^{-1}$)	73.3 \pm 51.6	61.0 \pm 49.0
Age at menarche (yr)	13.1 \pm 1.4	13.0 \pm 1.5
Total lifetime menstrual periods	68.5 \pm 27.9	67.4 \pm 30.0
History of menstrual irregularity	50.7%	64.2%
Current menstrual status		
Amenorrheic (0-3 periods in previous yr)	11.6%	6.2%
Oligomenorrheic (4-9 periods in previous yr)	18.8%	29.6%
Eumenorrheic (>9 periods in previous yr)	69.6%	64.2%
History of oral contraceptive use	43.5%	40.7%
Race/ethnicity		
White	82.6%	82.7%
Asian/Pacific Islander	4.4%	9.9%
Hispanic	7.2%	3.7%
Black	2.9%	0%
Other	2.9%	3.7%

*On a scale of 1-6: 1=never, 2=1-2 times, 3=3-5 times, 4=6-8 times, 5=9-11 times, 6=12+ times

†p=.03, groups differ by Wilcoxon test

‡Eating Disorder Inventory 0-69; 0=least disordered, 69=most disordered, Garner and Olmstead (1984)

•p=.08, Wilcoxon test

TABLE 2. Mean annual rate of change and standard error in body weight, fat mass, percent body fat, and lean mass by treatment randomization.

	Menstrual groups compared	OC group (N)	Control group (N)	OC mean minus control mean	p
Weight ($\text{kg}\cdot\text{year}^{-1} \pm \text{SE}$)*	all	-0.11 ± 0.30 (69)	0.43 ± 0.31 (81)	-0.54 ± 0.31	0.09
Fat mass ($\text{g}\cdot\text{year}^{-1} \pm \text{SE}$)**	all	-125.9 ± 241.0 (69)	223.2 ± 239.3 (81)	-349.1 ± 250.0	0.16
Percent body fat ($\%\cdot\text{year}^{-1} \pm \text{SE}$)**	all	-0.49 ± 0.31 (69)	0.09 ± 0.31 (81)	-0.58 ± 0.32	0.08
Lean mass ($\text{g}\cdot\text{year}^{-1} \pm \text{SE}$)***	irregular ¹ only	320.5 ± 289.5 (21)	304.3 ± 280.4 (29)	16.2 ± 346.7	0.96
Lean mass ($\text{g}\cdot\text{year}^{-1} \pm \text{SE}$)***	regular ² only	675.5 ± 154.3 (48)	-96.3 ± 137.5 (52)	771.8 ± 172.3	<0.0001

*From linear mixed models, adjusted for site, baseline menstrual status, and dieting frequency.

**Linear mixed models, adjusted for site and baseline menstrual status.

***Linear mixed models, adjusted for site.

¹<10 cycles in year prior to baseline

²10 or more cycles in year prior to baseline

TABLE 3. Mean differences in the adjusted annual rates of change in body measurements (OC minus control), by three criteria of actual oral contraceptive use.

	Menstrual groups compared	Used OCs 3+ months	p	Used OCs 6+ months	p	Proportion of time on OCs (per 100 percentage units)	p
Weight ($\text{kg}\cdot\text{year}^{-1} \pm \text{SE}$)*	all	-0.42 \pm 0.32	0.19	-0.51 \pm 0.31	0.10	-0.48 \pm 0.34	0.17
Fat mass ($\text{g}\cdot\text{year}^{-1} \pm \text{SE}$)**	all	-197.6 \pm 261.8	0.45	-173.0 \pm 253.7	0.50	-270.3 \pm 283.6	0.34
Percent body fat (%·year $^{-1} \pm \text{SE}$)**	all	-0.29 \pm 0.34	0.39	-0.28 \pm 0.33	0.39	-0.54 \pm 0.37	0.14
Lean mass ($\text{g}\cdot\text{year}^{-1} \pm \text{SE}$)***	irregular ¹ only	-254.9 \pm 349.0	0.47	-244.1 \pm 356.7	0.50	-110.5 \pm 429.0	0.80
Lean mass ($\text{g}\cdot\text{year}^{-1} \pm \text{SE}$)***	regular ² only	567.1 \pm 197.6	0.005	635.9 \pm 183.1	0.0007	825.8 \pm 197.0	<0.0001

*From linear mixed models, adjusted for site, baseline menstrual status, and dieting frequency.

**Linear mixed models, adjusted for site and baseline menstrual status.

***Linear mixed models, adjusted for site.

¹<10 cycles in year prior to baseline

²10 or more cycles in year prior to baseline

REFERENCES Note: Four duplicates below: 5=16, 6=21 ,15=20, 17=33, which will be corrected in the final draft.

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